High-Throughput Empirical Prediction of Nanoparticle Bioavailability, Toxicity, and Fate using Artificial Cell Membranes

Task 425.025: Development of Quantitative Structure-Activity Relationship for Prediction of Biological Effects of Nanoparticles Associated with Semiconductor Industries

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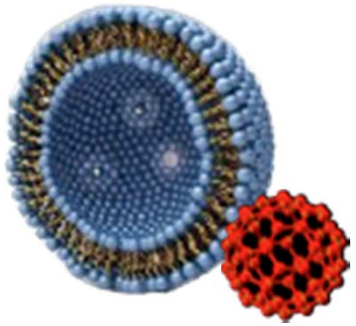

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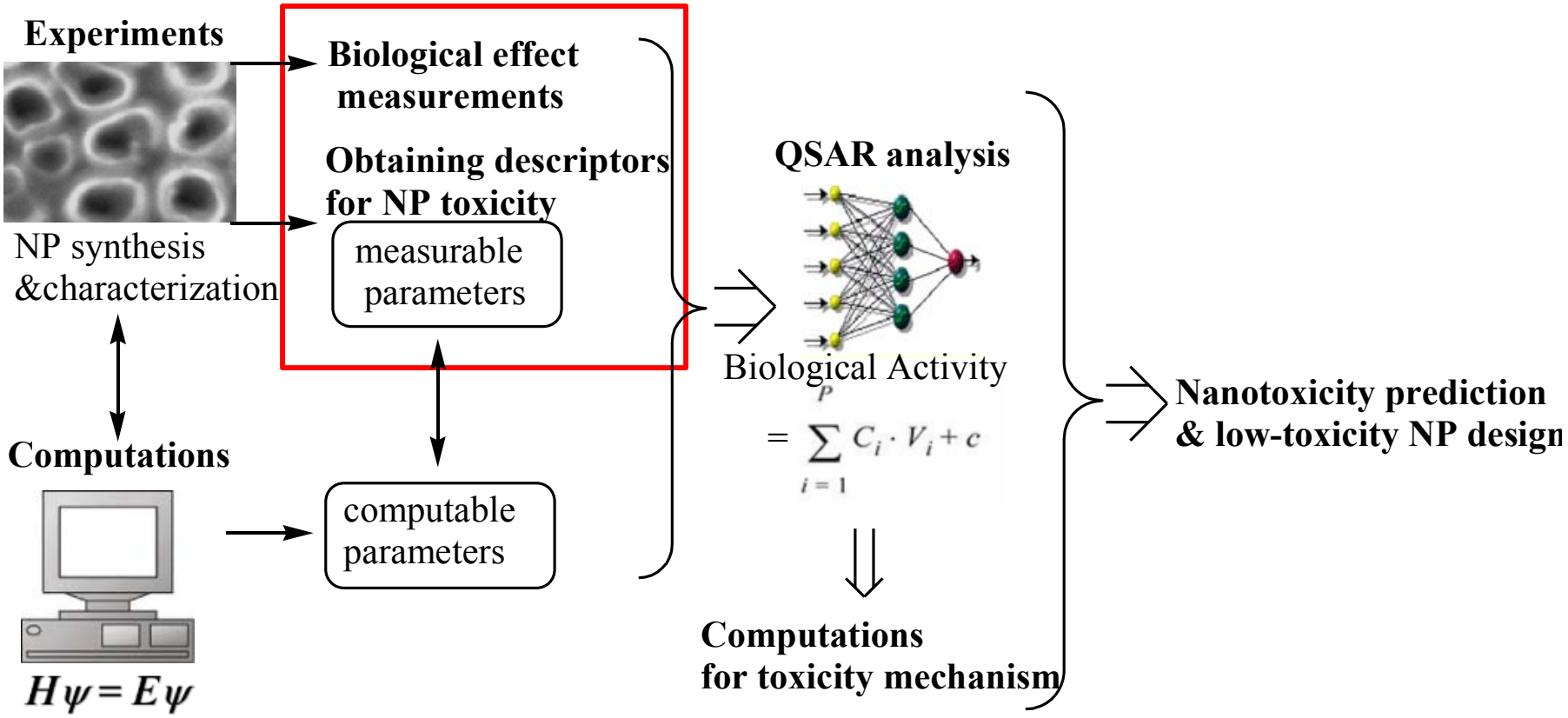
Rationale

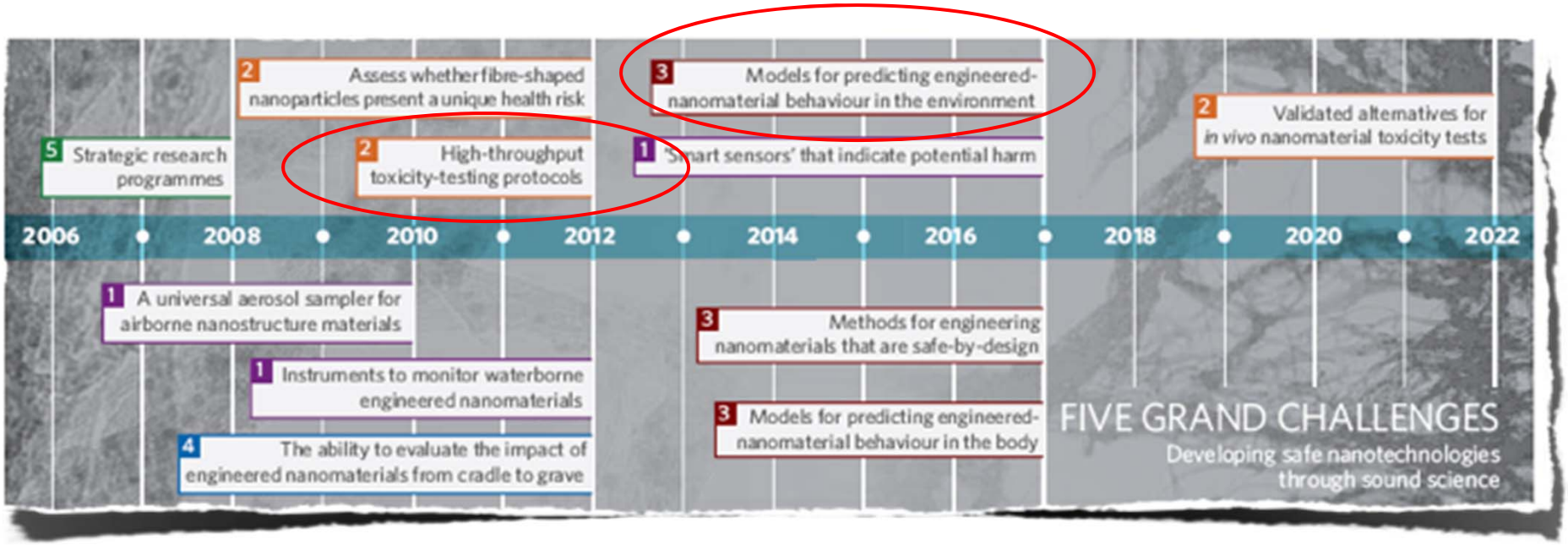
- ENMs will eventually be discharged into the environment at some point of their life cycle.
- The ultimate fate of these ENMs in the environment is still widely unknown and difficult to predict.
- A need exists for high-throughput empirical and modeling approaches to better understand and predict the bioaccumulation, toxicity, and distribution of ENMs among various environmental compartments (air, water, soil, and biota).

Quantitative Structure-Activity Relationship (QSAR) Model



Relates particle properties(direct or indirect) to biological effect (toxicity, bioaccumulation)

Grand Challenges



Maynard et al. , Safe Handling of Nanotechnology, Nature, 2006

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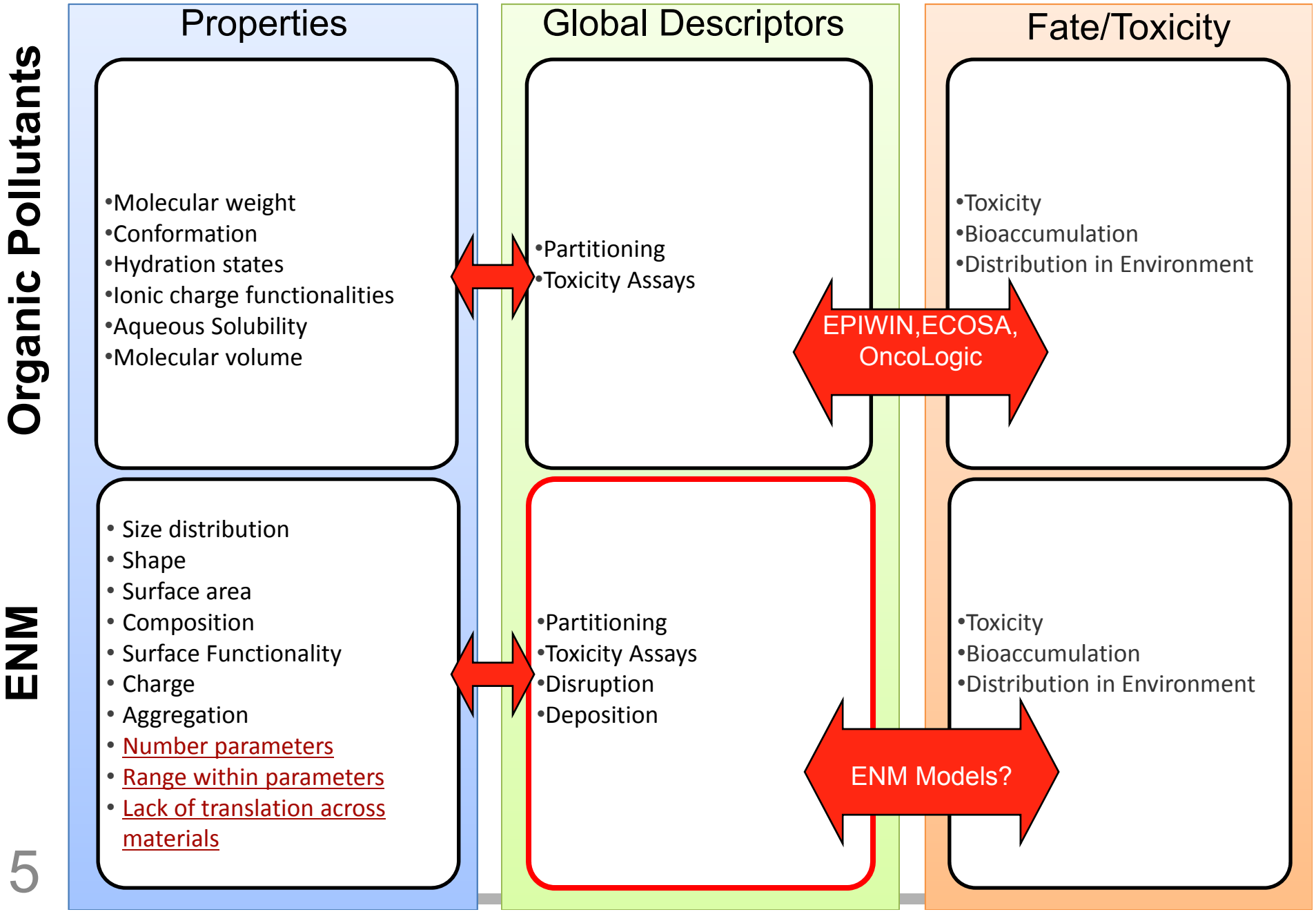
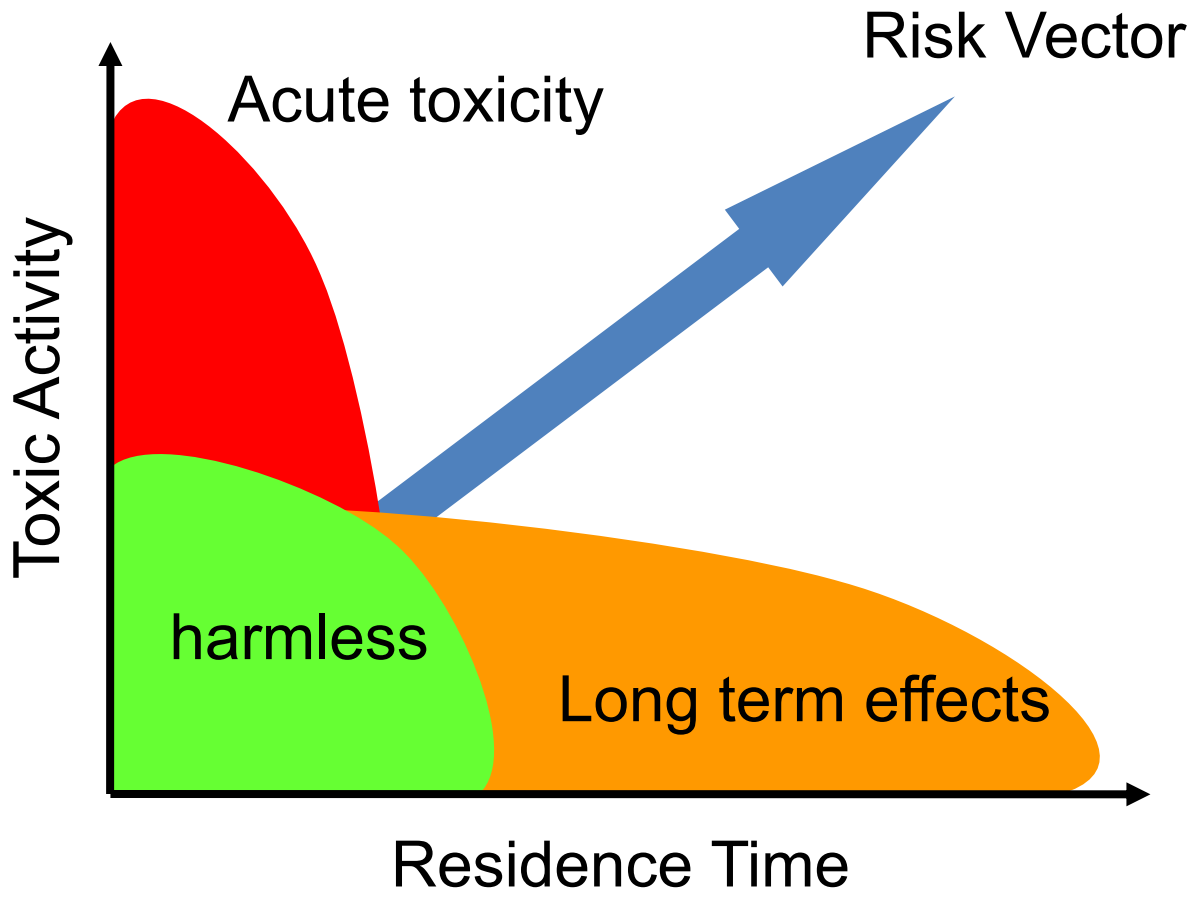
Using Global Descriptors to Predict ENM Fate, Toxicity & Transport

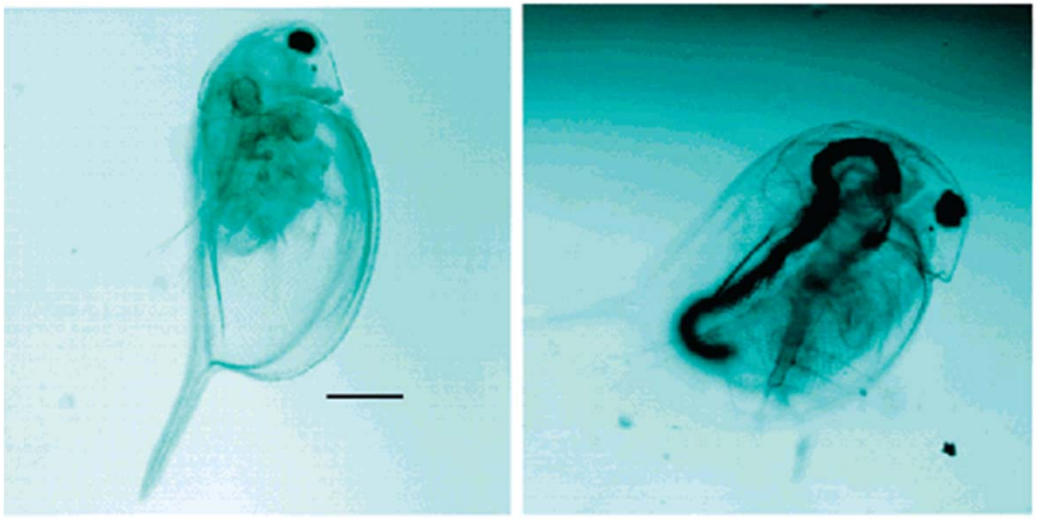


Toxicity Paradigm



- Risk ~ Occurrence Probability x Damage Potential
- We measure bioaccumulation which relates to occurrence probability
- We measure membrane disruption which relates to damage potential

Bioaccumulation of Nanoparticles in Organisms

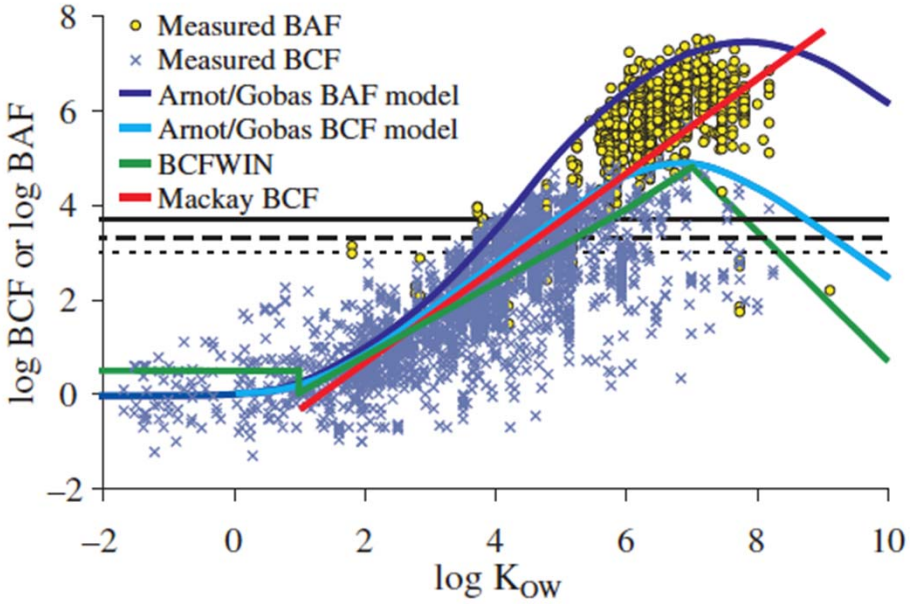


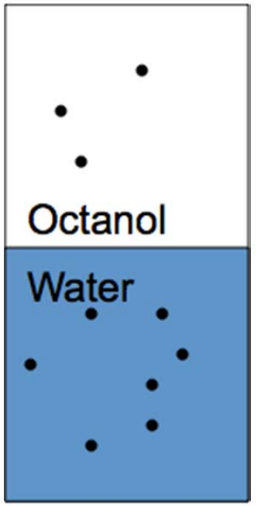
Single-walled carbon nanotubes accumulated in *Daphnia magna*. (Roberts et al., ES&T, 2007)

- Nanomaterials without acute toxicity will have to accumulate into organisms to exert toxicity.
- Increased exposure concentrations to some nanomaterials may cause enhanced toxicity to organisms. (Roberts et al., ES&T, 2007; Thill et al., ES&T, 2006; Tao et al., Chemosphere, 2010)

Bioaccumulation of Organic Contaminants is Largely Predicted by Octanol-Water Partitioning Coefficients

- Ratio of concentration of solute in between octanol and water.
- Used in water quality models (WASP, QUAL2K, Aquatox, EPD-RIV1) to predict fate, accumulation, aquatic toxicity of organic pollutants in the environment.
- Required for high-volume chemicals
- Methods published in OPPTS
- Methods most appropriate for unionizable chemicals such as many organic chemicals
- More difficult to interpret for ionizable substances
- Not defined for particles



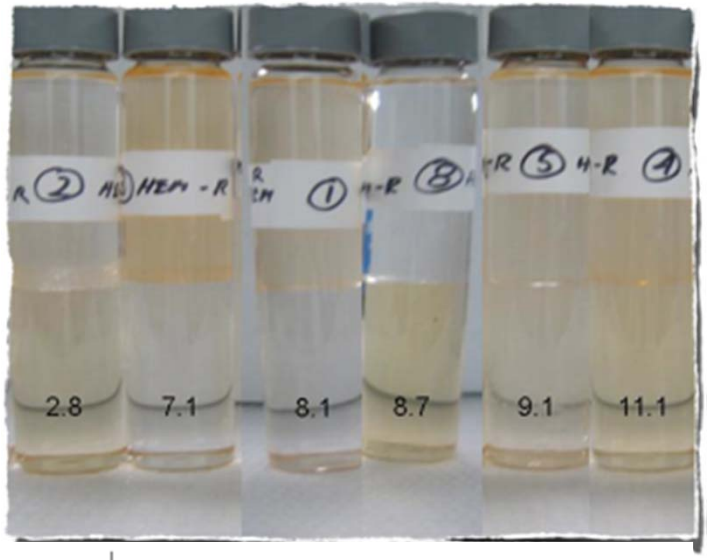


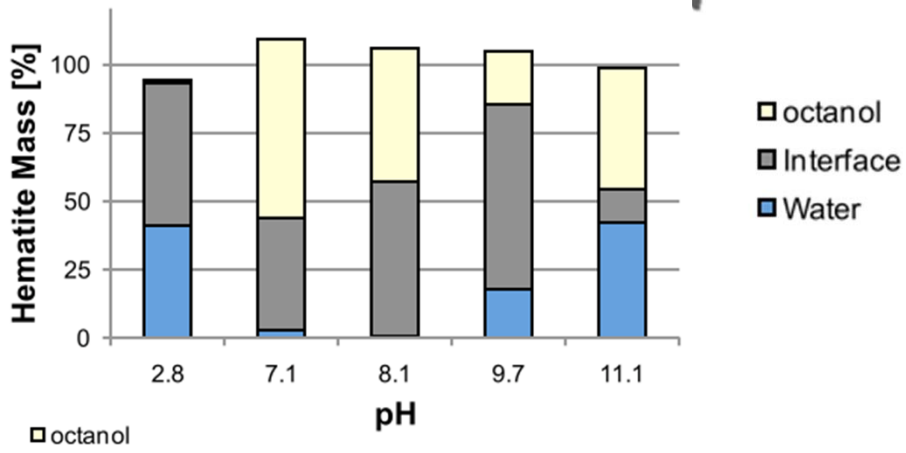
$$K_{ow} = \frac{C_{oct}}{C_w}$$

Octanol-Water Partitioning of ENM

- 60 mL Teflon cap glass vials
- 1 mM $NaHCO_3$ buffer (bicarbonate)
- Mixed for 3 days at 30 rpm
- Phases separated
- Vary pH, ionic strength
- high pH > 11 (NaOH)
- low pH < 3 (HNO_3)

Partitioning of Hematite Fe_2O_3

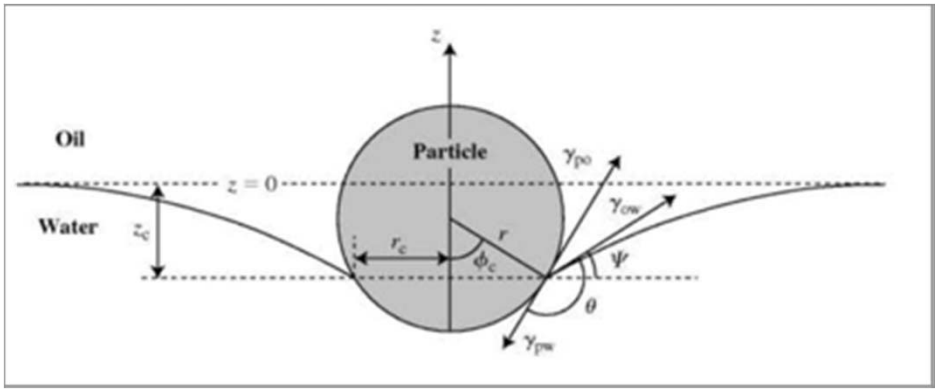




Octanol-Water Partitioning of ENM

ENM at Octanol-Water Interface

Minimum in Helmholtz Energy



$$E_0 - E_1 = \Delta E_1 = -\frac{\pi r^2}{\gamma_{ow}}\left[\gamma_{ow} - \left(\gamma_{pw} - \gamma_{po}\right)\right]^2$$

$$\gamma_{po} - \gamma_{pw} = \gamma_{ow}\cos\theta_{ow}$$

- Some particles at interface
- Interface area and properties poorly characterized
- Not quantified or treated in classical partitioning theory
- NP octanol-water coefficients do not predict BCF

*Petersen et al. Relevance of Octanol-Water Distribution Measurements to the potential Ecological Uptake of Multi-Walled Carbon Nanotubes, Environmental Toxicology and Chemsitry, 2010

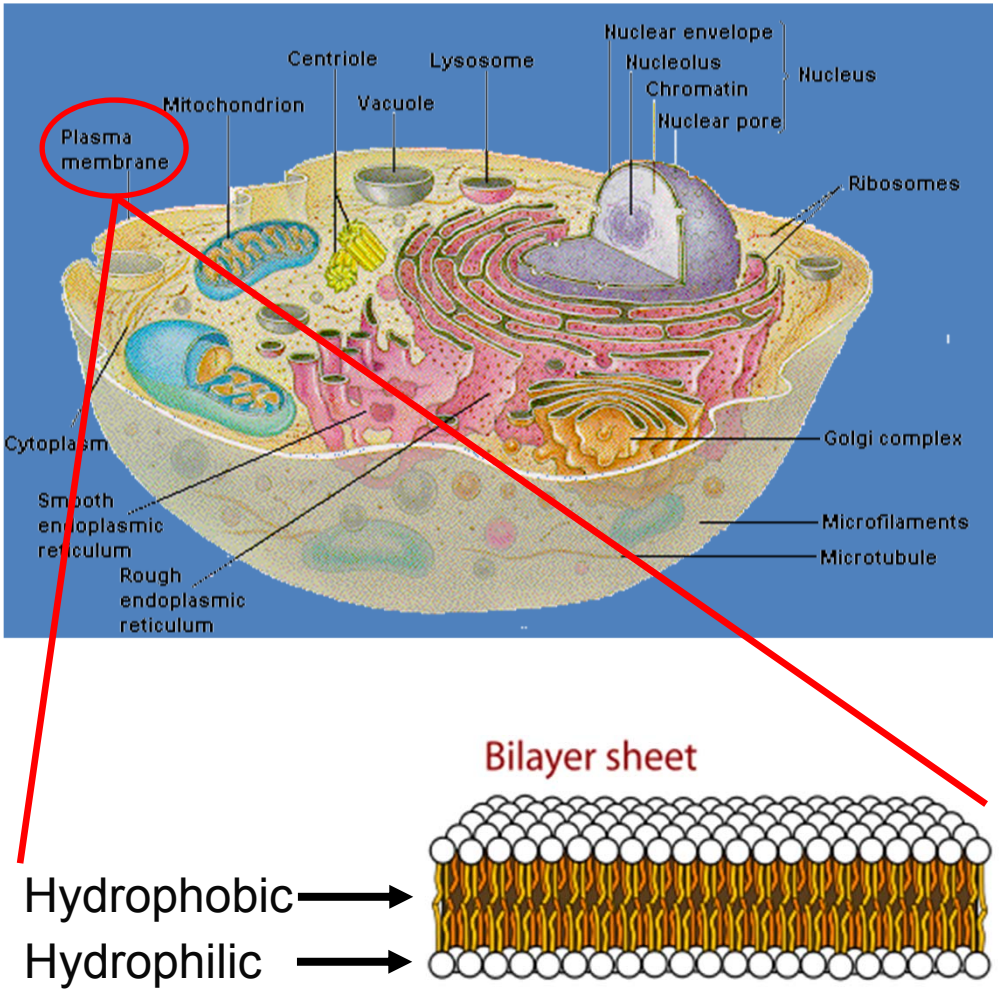
Current Efforts

- High throughput empirical measurements for prediction of NP bioaccumulation using lipid-water distributions.
- High throughput empirical measurements of toxicity potential using disruption of membranes.
- Experiments give scientific insight into mechanisms of interaction between NPs and biological interfaces

Lipid Bilayers as Model Cell Membranes

- Lipid bilayers are the primary constituents of many biological cellular membranes. Arguably the most important interface between cellular life and its surrounding environment.

- Lipid - amphiphilic molecule that can spontaneously arrange in aqueous solution to have a hydrophobic interior and hydrophilic exterior.



ENM Distributions in Lipid-Water Systems

- Lipid bilayer is an important interfaces between life and its environment and a potential exposure route to ENMs.
- The lipid bilayer-water distribution (K_{lipw}) has been shown to be a more appropriate indicator than (K_{ow}) for bioaccumulation of ionizable organic molecular and surface active compounds, which ENMs share some properties (e.g., charged surface and residence in interface).
- K_{lipw} is increasingly used by the pharmaceutical industry and environmental research for drugs and molecular pollutants.
- All mass is at the surface
- Surface area can be controlled and quantified
- High-throughput

Platforms for Quantifying Nanoparticle Interactions with Model Cell Membranes

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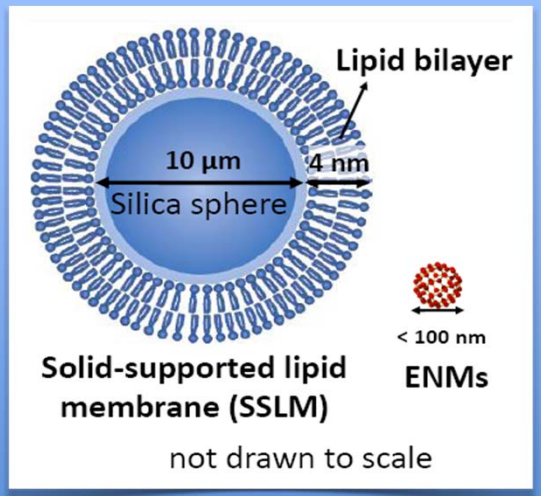

Adsorption to Bilayers to Predict Bioaccumulation Potential

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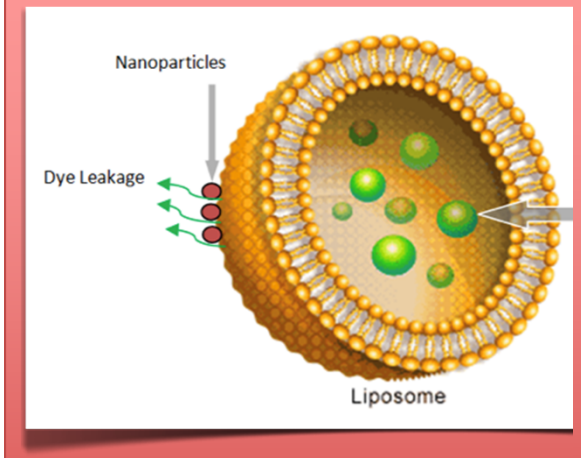

Dye Leakage through Liposomal Lipid Bilayers to Predict Toxicity Potential

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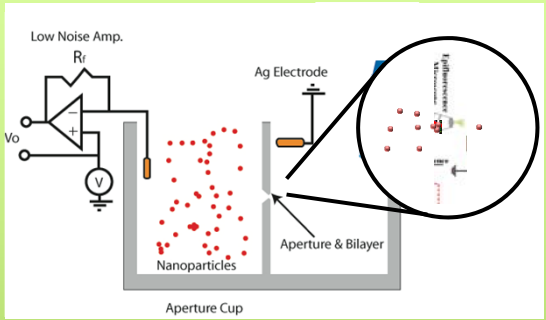
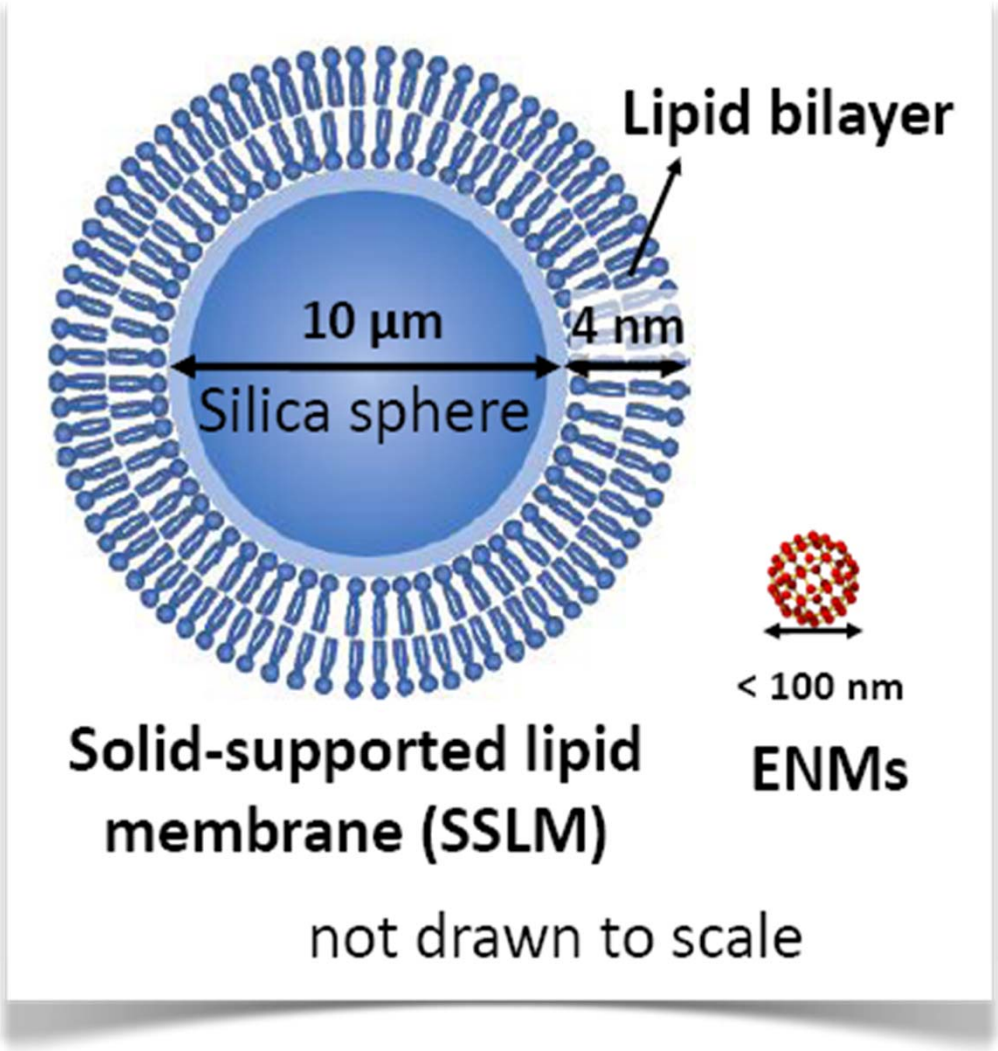
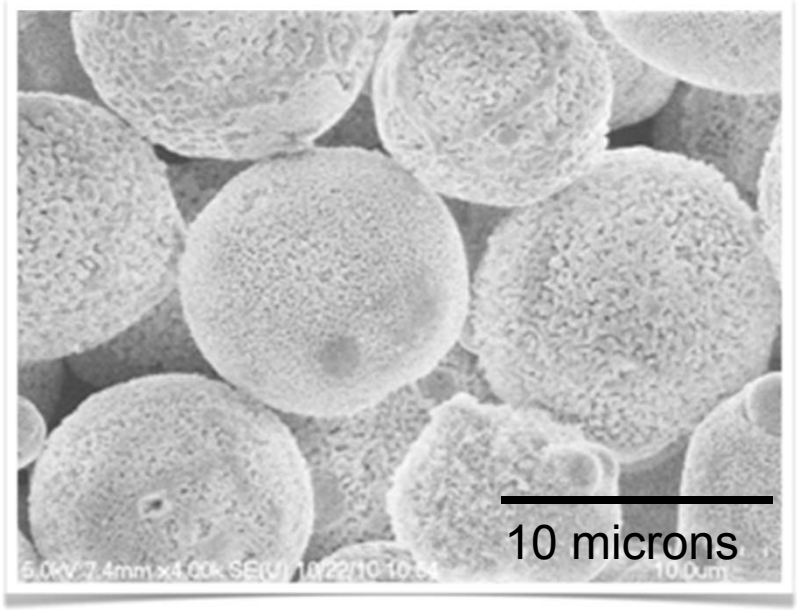


Ion Current Leakage through Suspended Bilayers to Predict Toxicity Potential

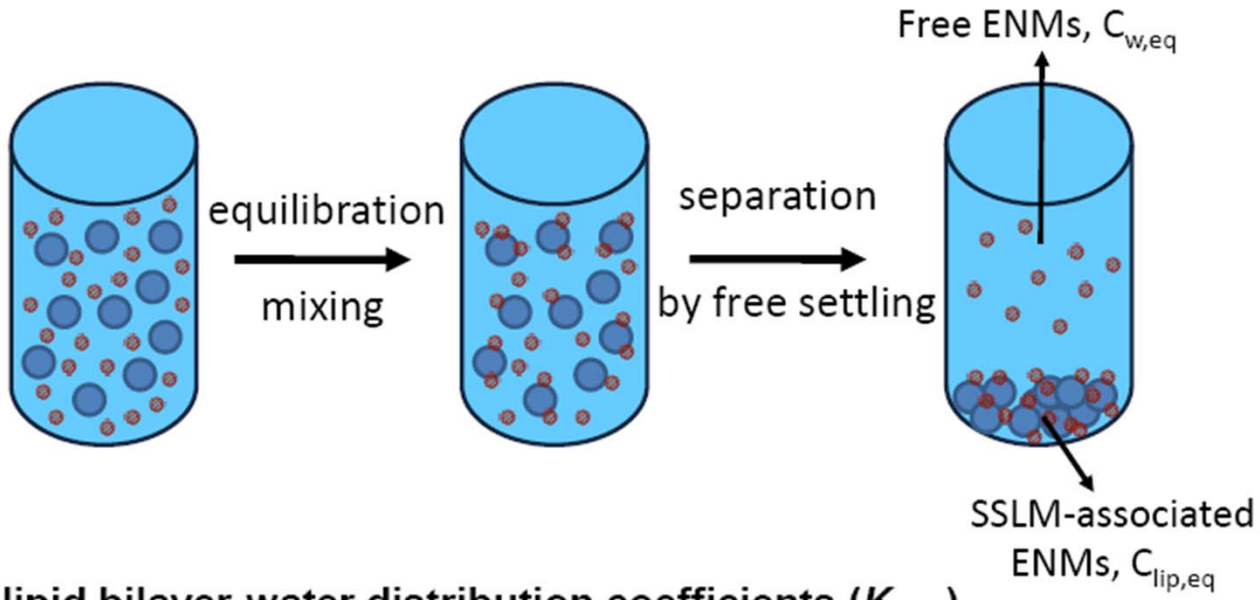
Lipid bilayer-water distribution on Solid Supported Lipid Bilayers





- Lipid Bilayer noncovalent bond to silica
- Bilayer is fluid
- Bilayer robust over wide range electrolyte conditions

Lipid Bilayer-Water Distribution of ENMs Method



Calculation of lipid bilayer-water distribution coefficients (K_{lipw})

$$K_{lipw} = \frac{C_{lip,eq}}{C_{w,eq}}(L/kg) \quad \text{where}$$

$$C_{lip,eq} = \frac{(C_{w,ref} - C_{w,eq})(mg/L)}{m_{lip}(kg/L)}$$

$C_{w,ref}$ = [ENMs] in the control samples

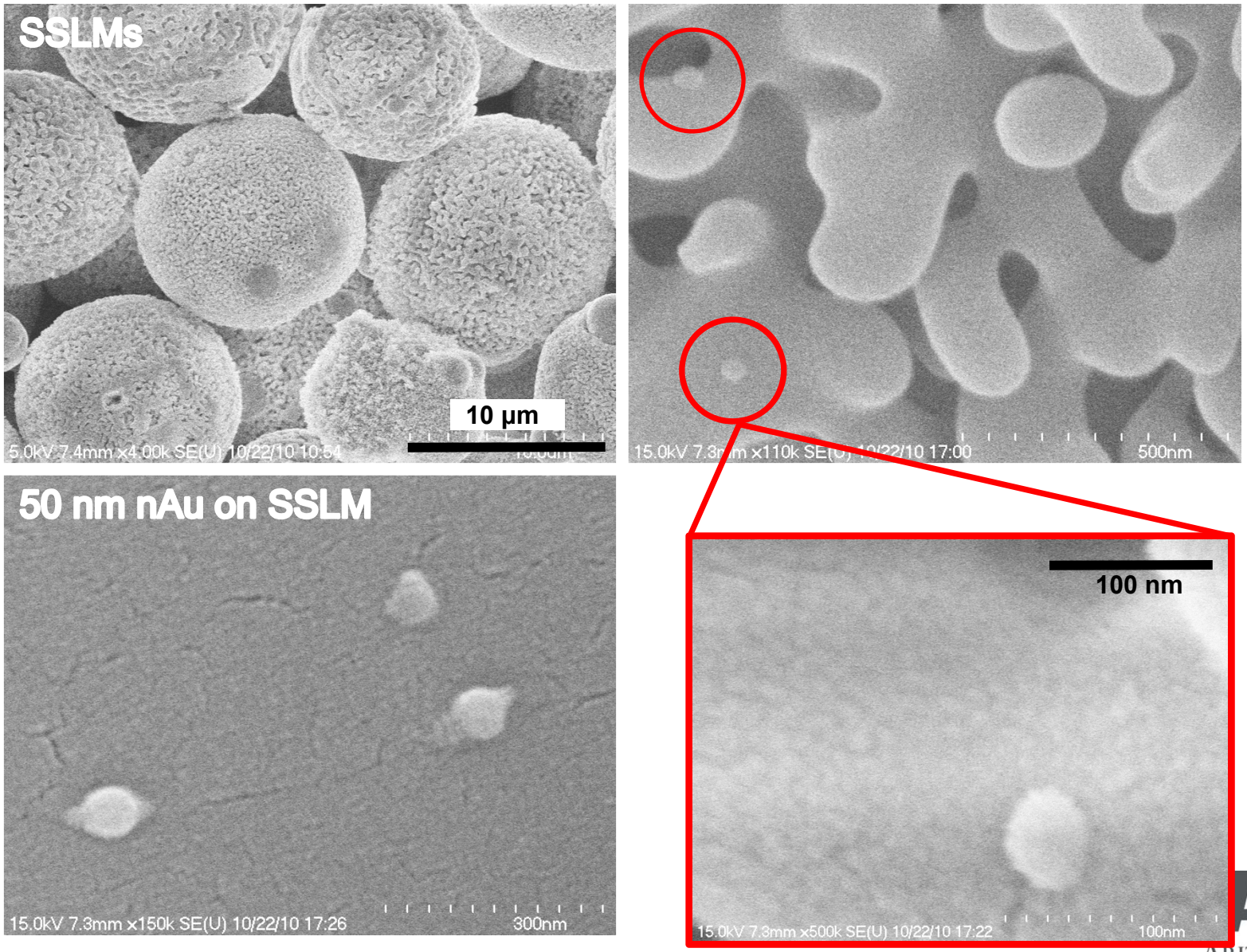
$C_{w,eq}$ = free [ENMs] in supernatants at equilibrium

m_{lip} = lipid concentration

Validated using a reference compound (2,4,6-trichlorophenol).

log K_{lipw} = 3.89 ± 0.03 (L/kg), close to 3.90 reported by Escher et al. (ES&T, 1996)

SEM Images of NP Adsorbed on SSLMs



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ENMs and Lipid Sources and Preparations

ENMs:

- **Aqueous C_{60} aggregates (nC_{60})**: dry C_{60} powders (MER, Tucson, AZ) were pulverized and then mixed with DI water for 2 weeks prior to passing through 0.7 and 0.45 μm filters sequentially (Hou et al., 2009).
- **Fullerol ($C_{60}(ONa)_x(OH)_y$, x + y = 24)**: dry fullerol powder (MER, Tucson, AZ) was directly mixed with water and then passed through 0.7, 0.45, and 0.2 μm filters sequentially.
- **Gold nanoparticles (nAu)** were tannic acid coated and well-characterized gold colloids at 5, 10, 20, 50, and 70 nm, purchasing from nanoComposix (San Diego, CA).
- **Cerium Oxide (CeO_2)** round-robin colloids at ~100 nm, provided by G. Tech and purchased from Sigma-Aldrich.

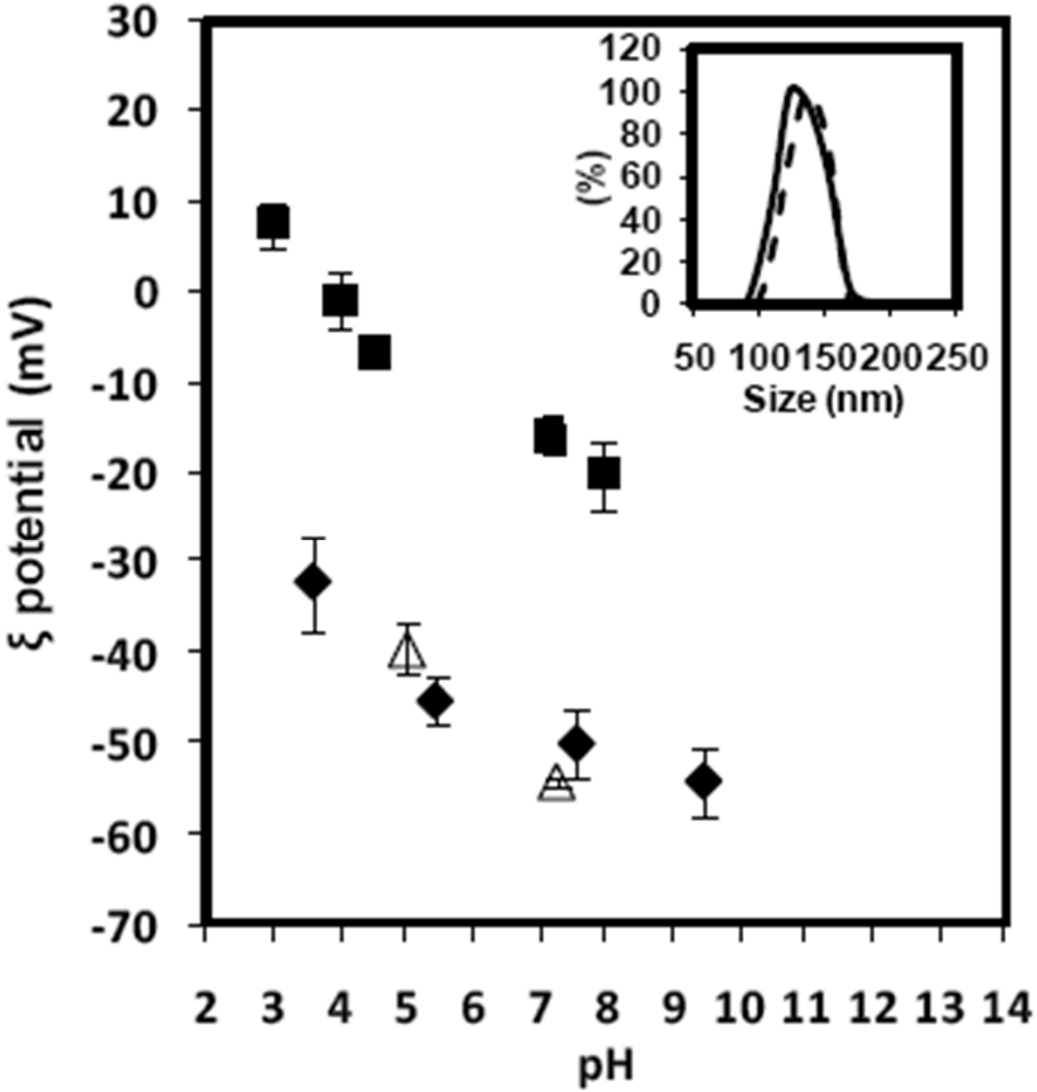
Lipid bilayers:

- SSLMs were purchased from Sovicell (Leipzig, Germany). The lipid composition was 100% phosphatidylcholine from chicken egg.
- Unilamellar lipid bilayer vesicles (i.e., liposomes) were prepared using the same lipid composition as SSLMs by the extrusion method (Hope et al., 1985). Liposomes were used to determined the effective zeta potential of lipid bilayers.

Analytical methods

- **nC_{60} concentration** was determined by high performance liquid chromatography (HPLC) with UV detection at 336 nm. Because HPLC is only applicable to molecules, molecular C_{60} was extracted from the aqueous phase to toluene in the aid of 0.1 M $Mg(ClO_4)_2$. The toluene extract was injected to HPLC (Hou et al., 2009).
- **Fullerol concentration** was determined by UV-visible absorption spectroscopy using UV at 254 nm.
- **nAu concentration** was determined by inductively coupled plasma-optical emission spectroscopy (ICP-OES). Prior to ICP, nAu was dissolved in aqua regia (i.e., 1 part of HNO_3 and 3 parts of HCl).
- **Cerium Oxide** concentration determine by ICP-OES. Prior to ICP, CeO_2 was digested in heated HNO_3.
- **Lipid concentration** was determined by the malachite green dye assay (Petitou et al., 1978). Before assay, lipid was digested, adding concentrated H_2SO_4 and H_2O_2 under heating.
- **The sizes and zeta potential** of liposomes and ENMs were determined by dynamic light scattering (DLS) (NICOMP 380 ZLS, Particle Sizing Systems, Santa Barbara, CA).

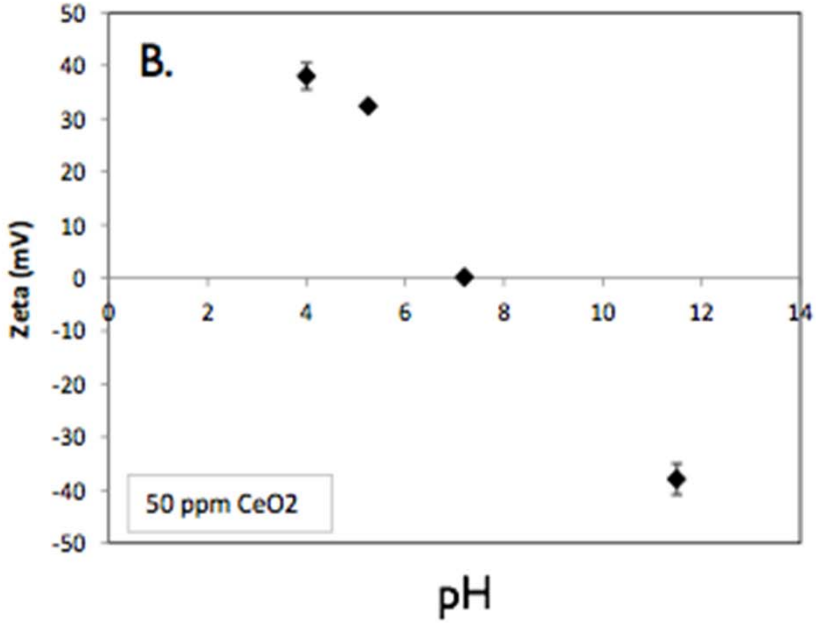
nC60 and Fullerols Size and Effective Zeta



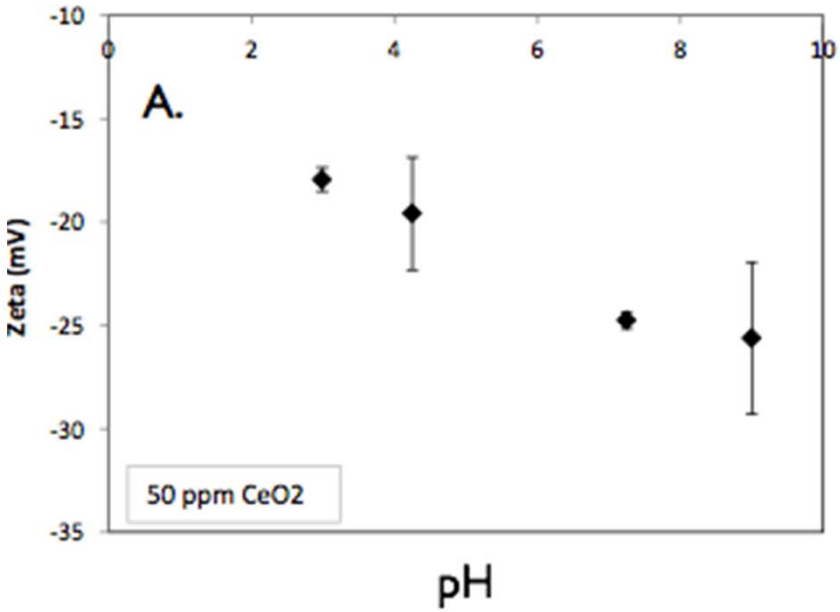
Lipid bilayer vesicles (■)
Fullerol 650 mg/L (♦)
nC_{60} 6.5 mg/L (Δ)

nC60 and fullerols have similar size distributions and charge

Cerium Zeta & Size

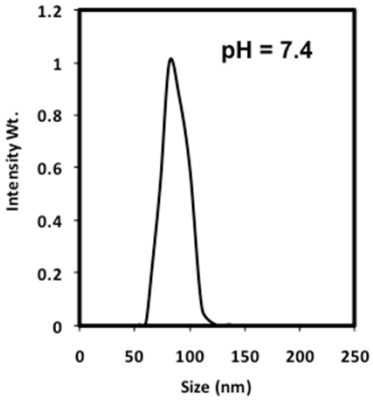


DI w/ NaOH and HCl



5 mM Phosphate

- Positively charged at low pH
- Isoelectric point is pH~7
- Negatively charged in phosphate buffer at all pHs (preferential adsoprtion)
- Phosphate tends not to be a good buffer



1000 ppm

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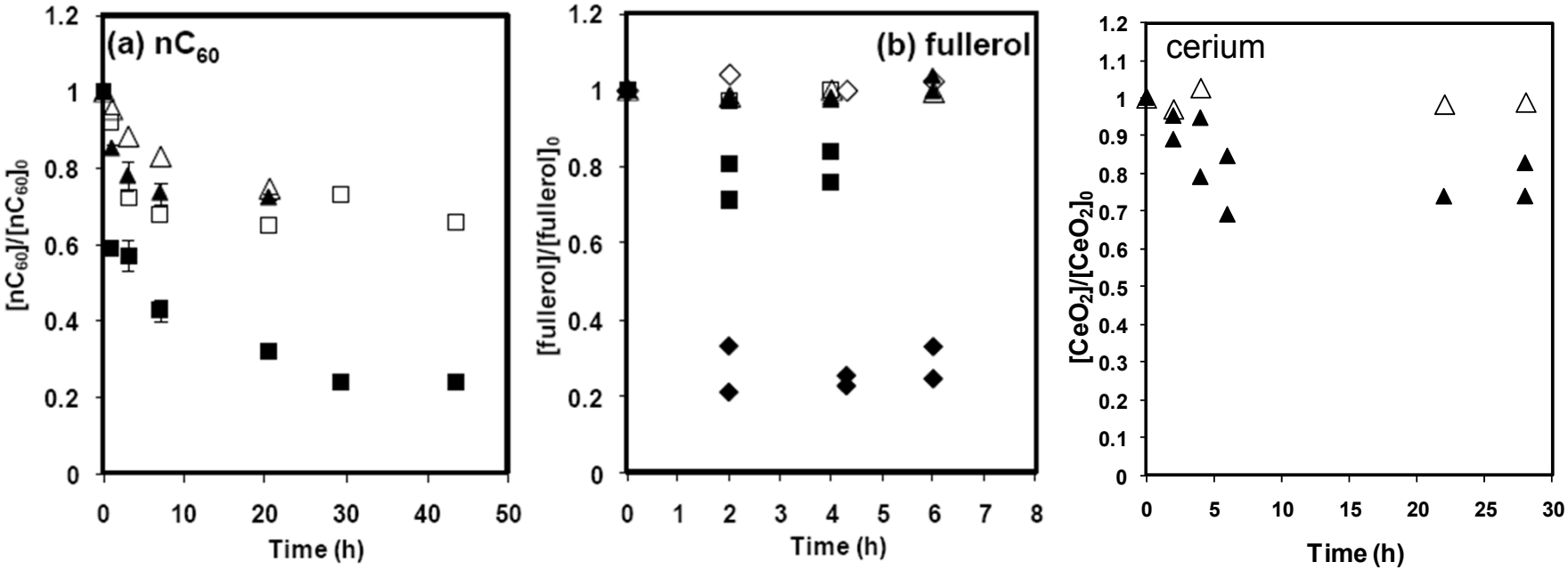
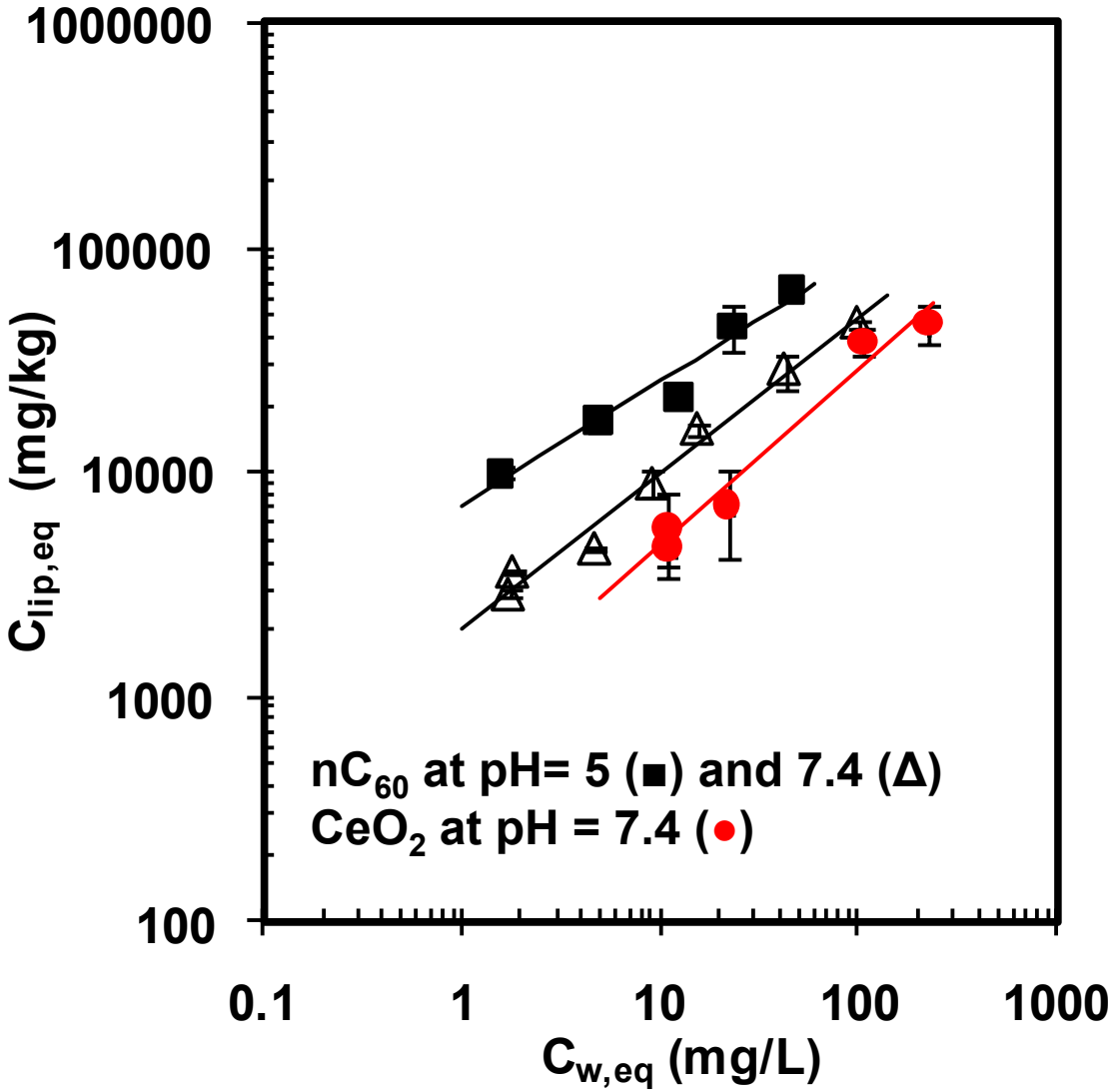
Nanoparticle Lipid-Bilayer Interaction Kinetics



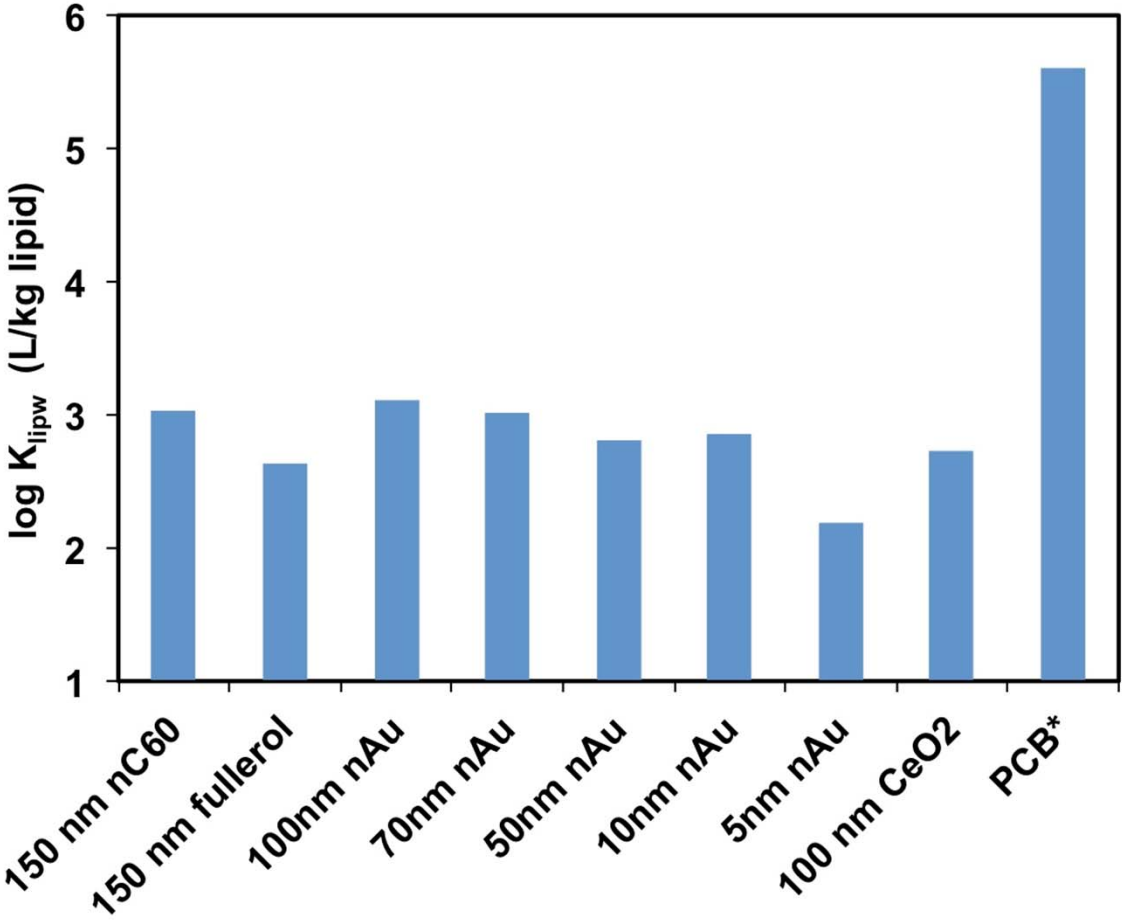
pH=3 (♦) and control (◊) pH=5 (■) and control (□) pH=7.4 (▲) and control (Δ)

- nC60 reach equilibration in 20-30 hours
- Fullerol reach equilibration in <3 hours
- Cerium reach equilibrium in <5 hours
- [lipid] = 0.47 mM
- Controls are vial without SSLM
- $[nC_{60}]_0$ = 6.5 mg/L;; $[fullerol]_0$=8.0 mg/L; $[CeO_2]$ = 14 mg/L

Lipid-Water Distribution Isotherms

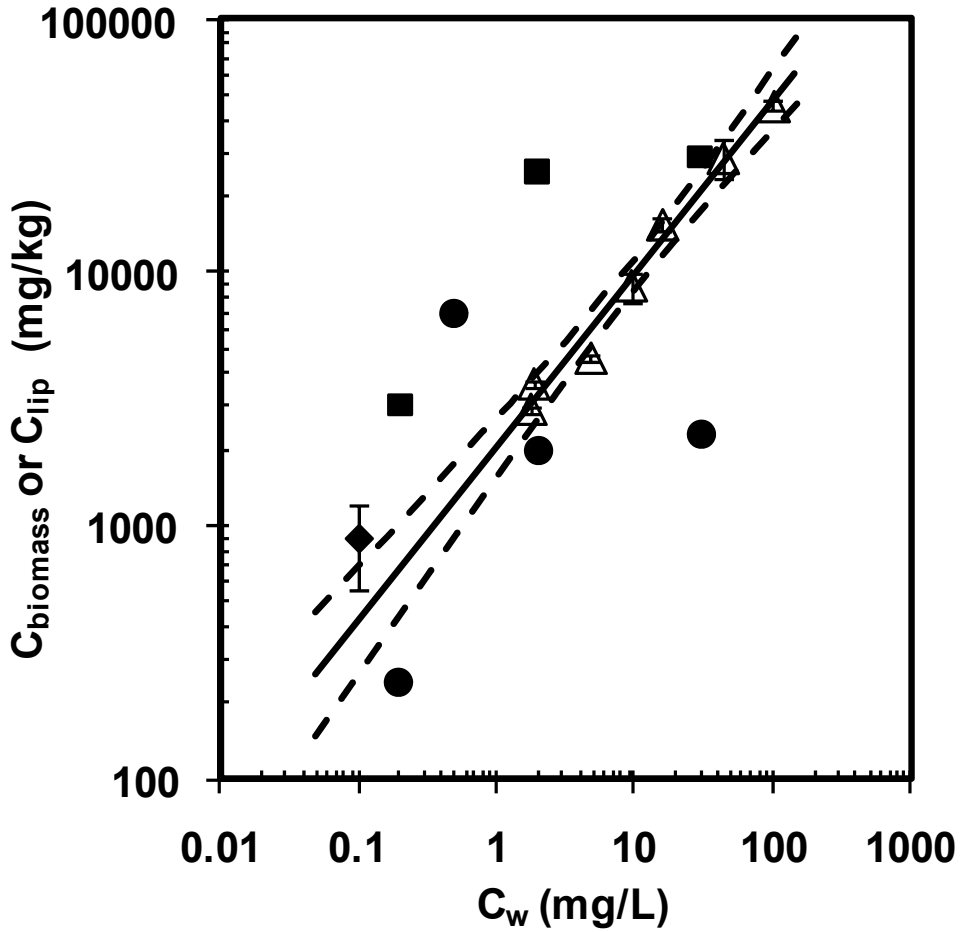


Lipid-Water Distribution Coefficients



- NP pK_{lipw} depends on size & charge & varies from 3-2.5
- PCB pK_{lipw} varies from 4.7-6.6
- PCB* is 2,2',3,3'-tetrachloro biphenyl
- Estimated from Freundlich isotherm fits at 5 mg/L

Bioaccumulation of Nanoparticles



- Comparison between K_{lw} nc60 bioaccumulation studies on daphnia magna
- Lipid-water may be good predictors of NP BCF

Tervonen et al.,*Environ Toxicol. Chem.* **2010**, 1072-1078; Tao et al., *Chemosphere* **2009**, *77*, 1482–1487; Oberdörster et al., *Carbon* **2006**, *44*, 1112–1120.

Summary of Lipid-Water Distributions

- The lipid bilayer-water distribution of the selected ENMs is pseudo-equilibrium process that can be described by isotherm behaviors.
- Accumulation to lipid bilayers increasing as pH dependent (electrostatics) analogous to ionizable organic pollutants.
- Comparisons with bioaccumulation suggest that the lipid bilayer-water distribution is promising for assessing the bioaccumulation and toxicity potentials of ENMs.

Bilayer Leakage Assays to Predict Toxicity Potential

- Measure of disruption of bilayers caused by NP
- More efficient than in-vitro or animal testing
- Potential to predict (global descriptor) toxicity
- Potential for high-throughput
- Each technique has range of sensitivities and limits.

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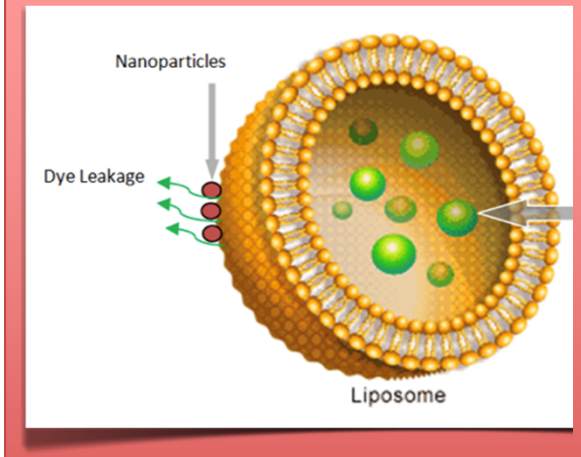

Dye Leakage through Liposomal Lipid Bilayers to Predict Toxicity Potential

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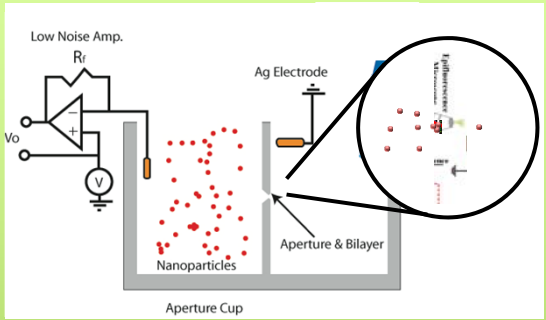
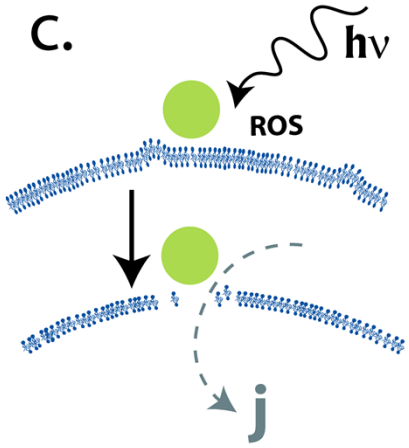


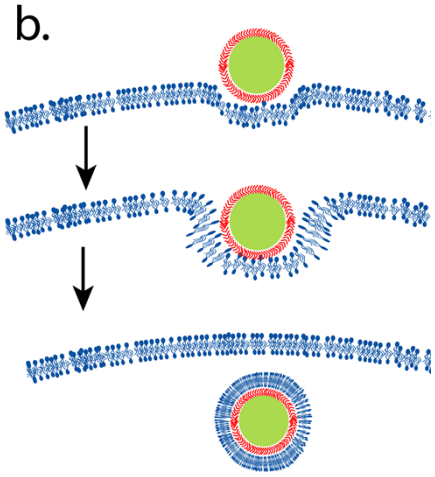
Ion Current Leakage through Suspended Bilayers to Predict Toxicity Potential

Interactions at BILAYER Interfaces

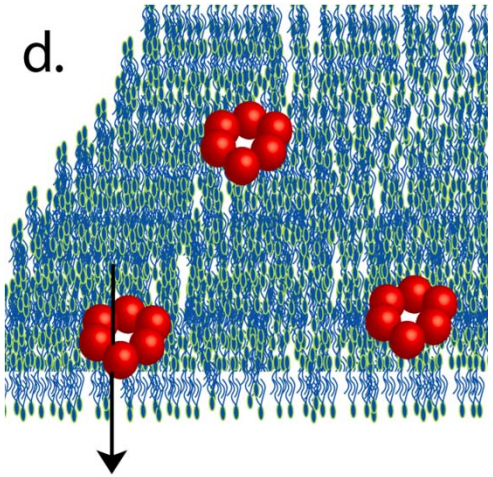
Variety of NP interactions with bilayers are possible that result in leakage.



ROS Induced

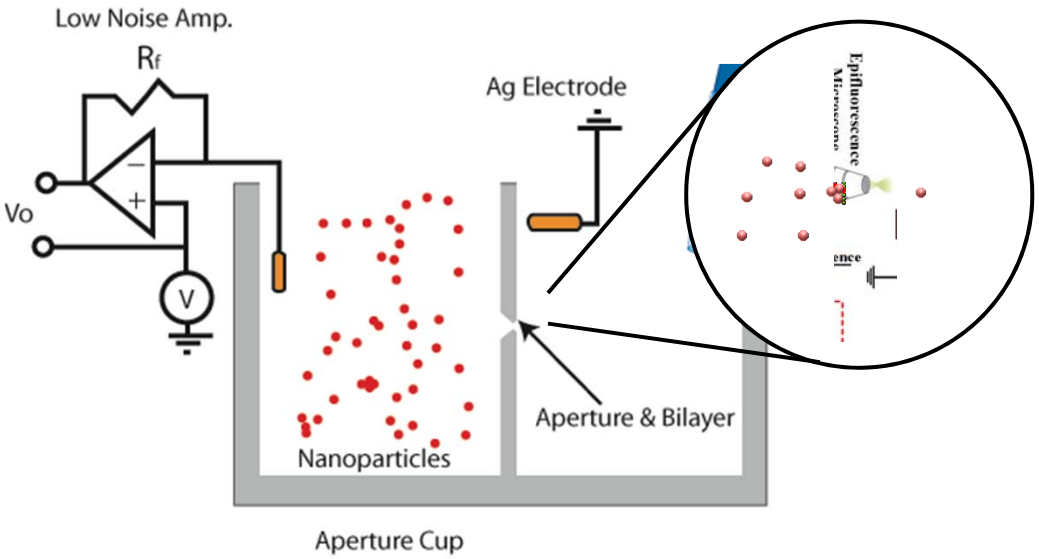


Unmediated Endocytosis



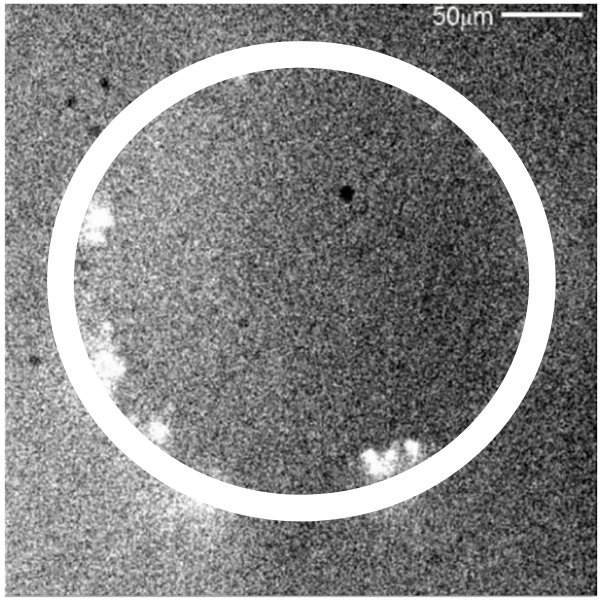
Self-Assembly

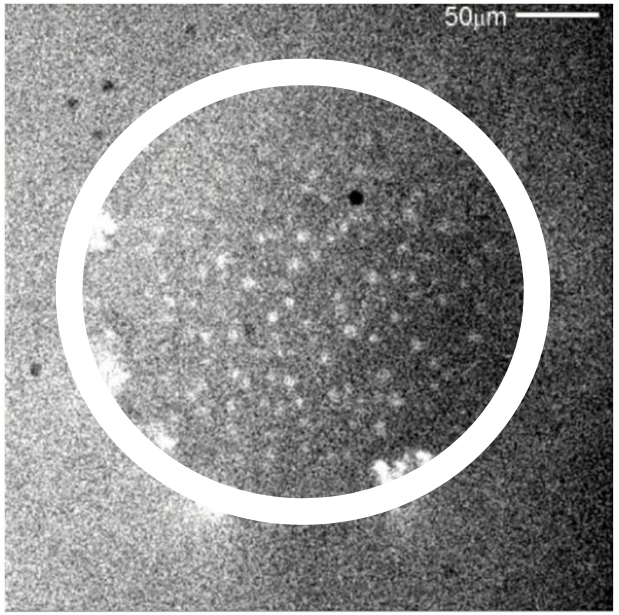
Suspended Bilayer (BLM) Electrophysiology Measurements



- 150 uM aperture Warner bilayer cup (3 ml)
- Heka Extracellular Patch Clamp Amplifier
- DOPC lipids
- Ag/AgCl electrodes
- 20 mM HEPES pH 7.4

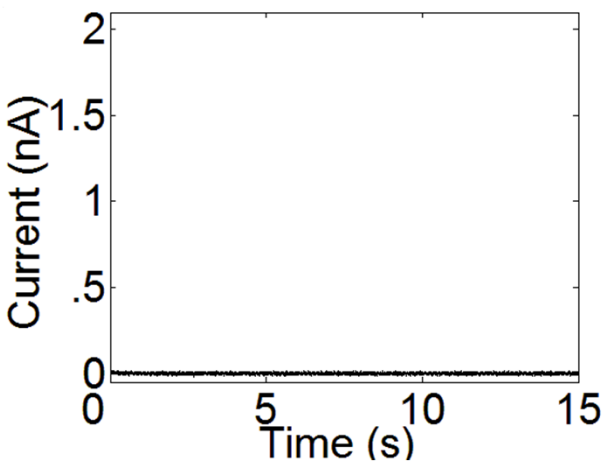
- Ionic Strength:
  - High ionic strength typically required for electrophysiology results in aggregation
  - Found that 20 mM KCL and 20 mM (pH=7.4) resulted in adequate SNR and minimized aggregation

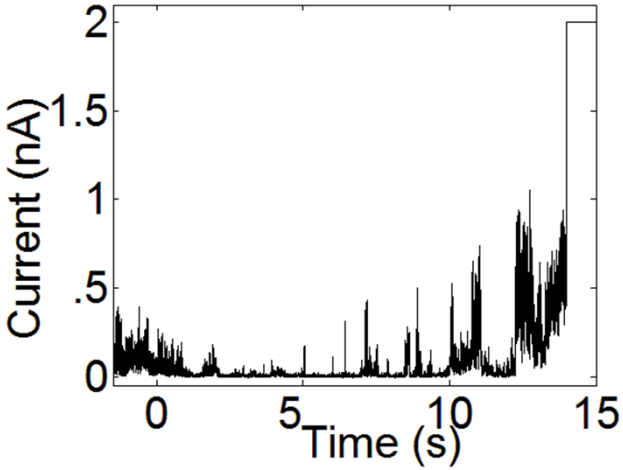




Before QDs are added

QD aggregation on bilayer





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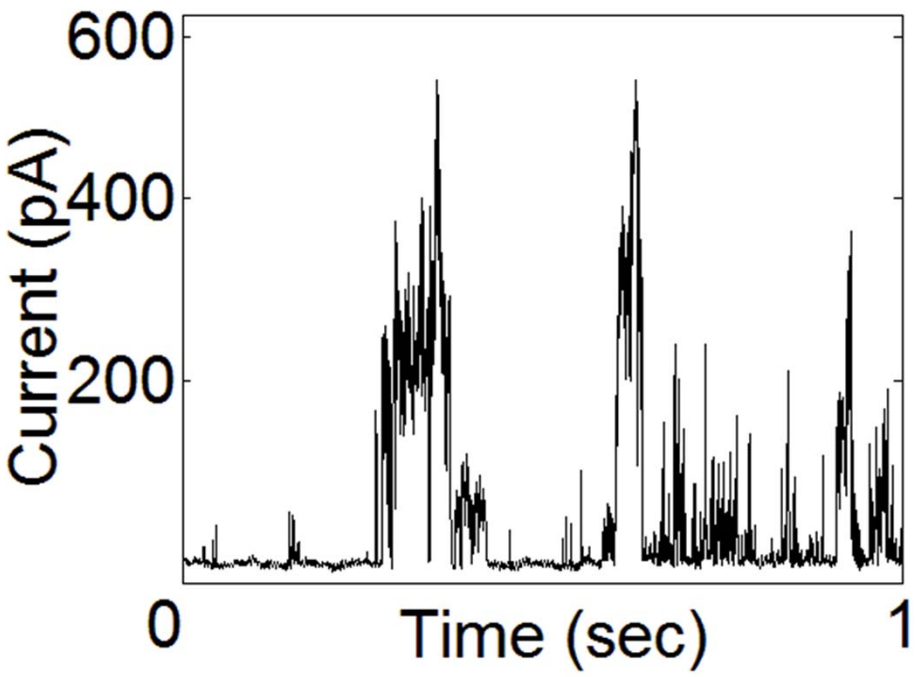
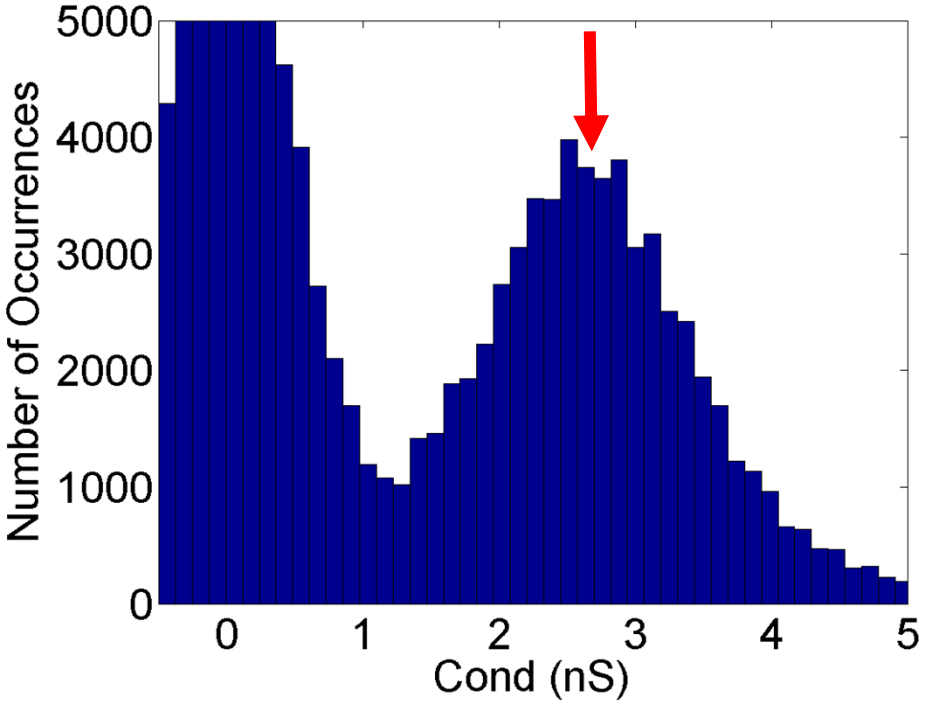
Conductance Measurements

Current Trace

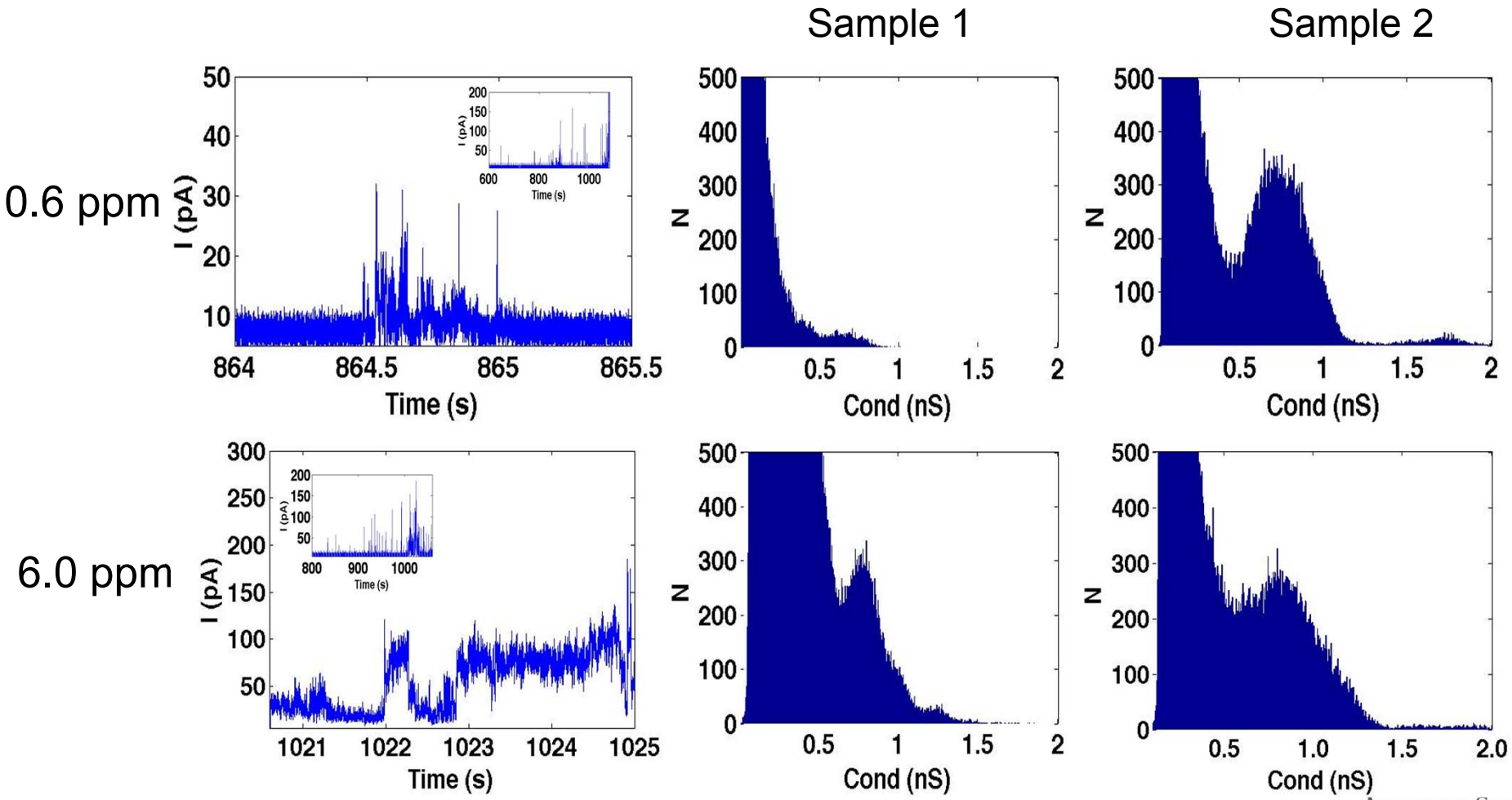


Conductance Histogram



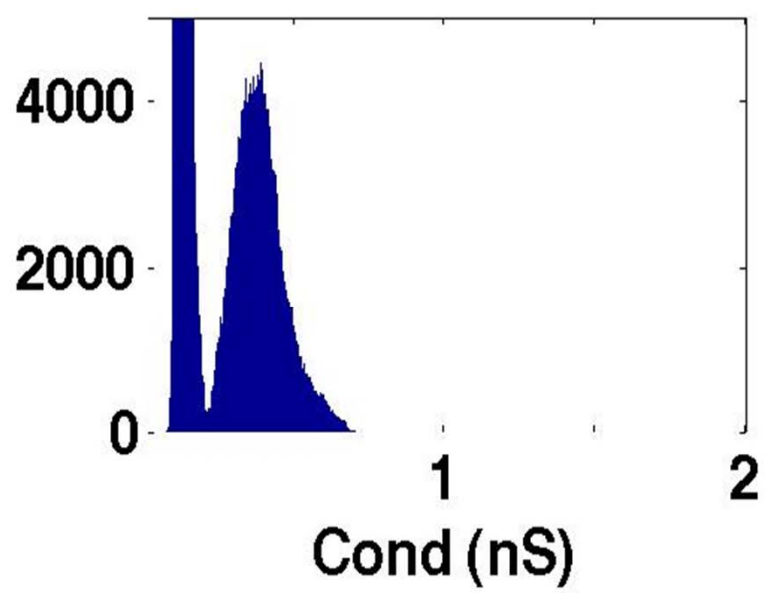
Reference NP: CdSe Core QD

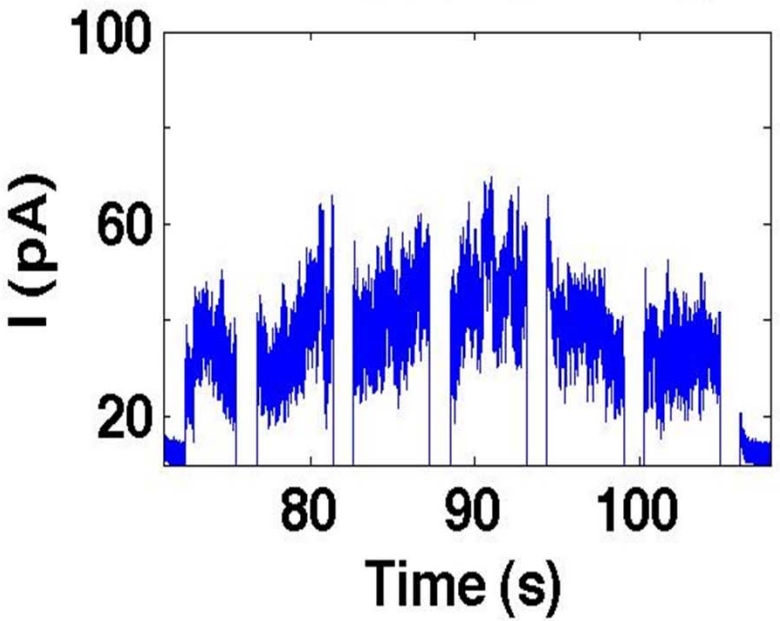
- Reference NP selected due to strong, repeatable interaction with bilayer
- CdSe Shell, ZnS Shell, Carboxyl Coating (Qdot® 525ITK™)
- Positive experiments 13/16 = 81%



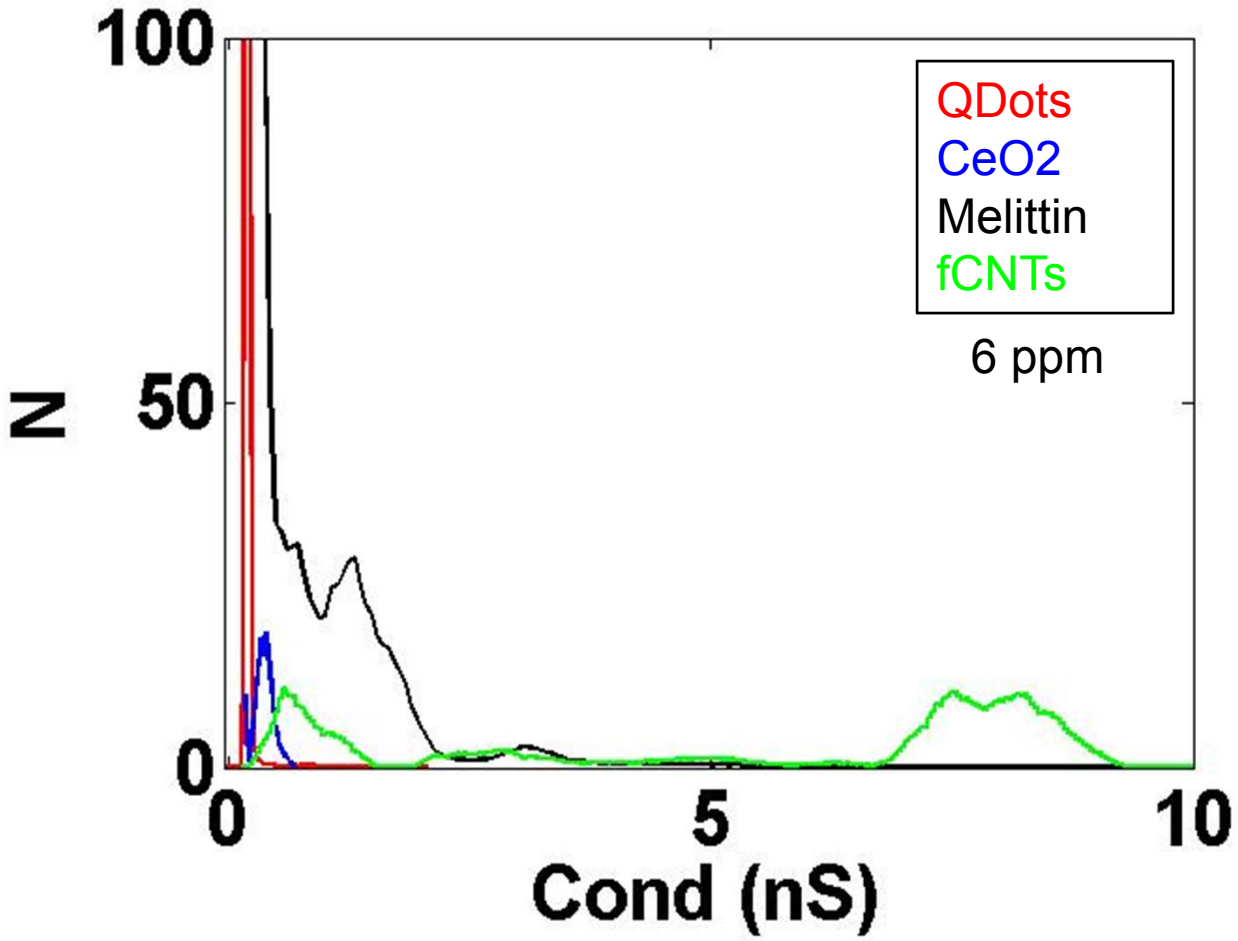
Cerium

6.0 ppm





Leakage Comparison



- Cerium induces small leakage compared to toxin melittin
- Functionalized CNT induce large leakage

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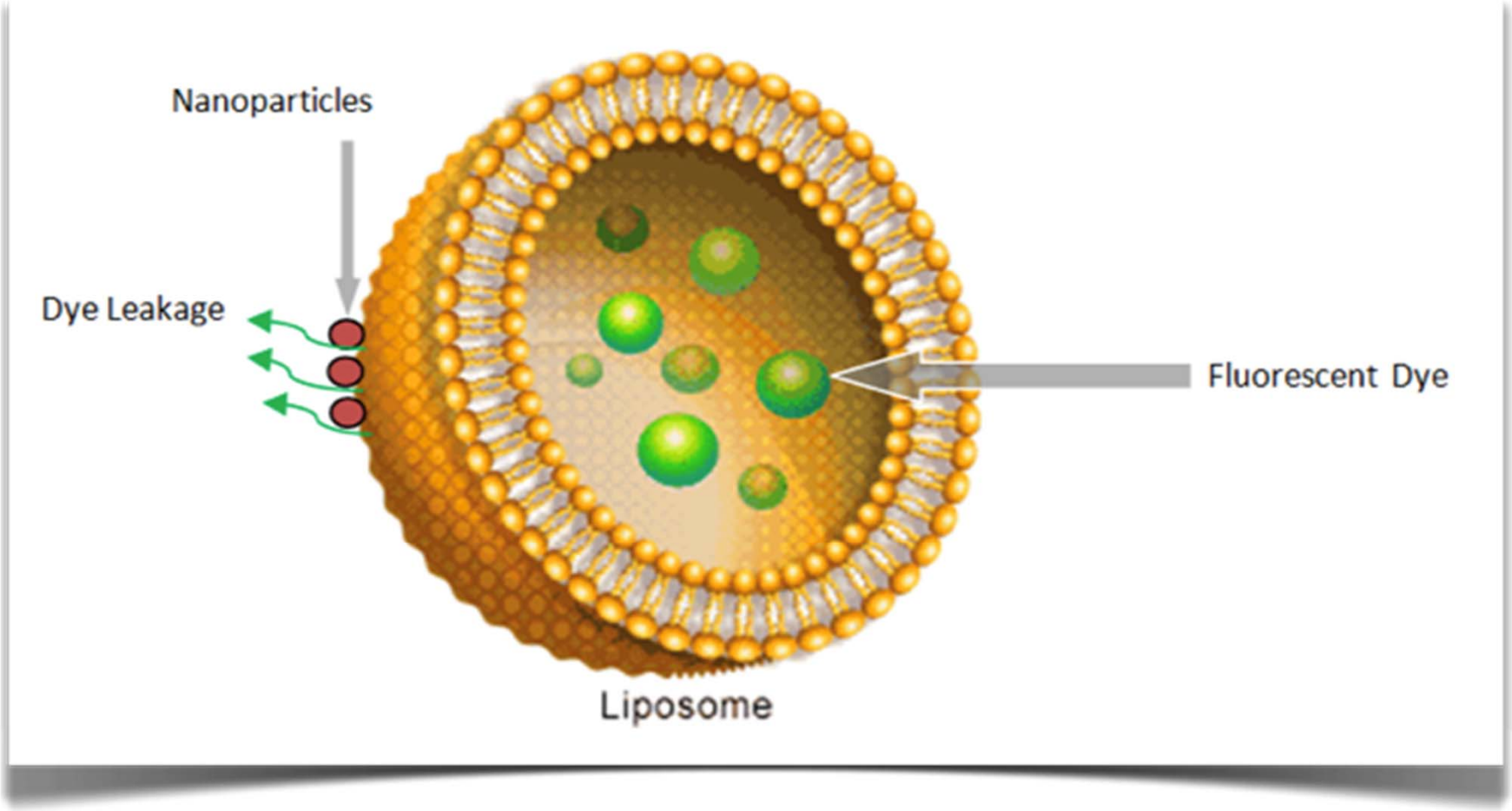
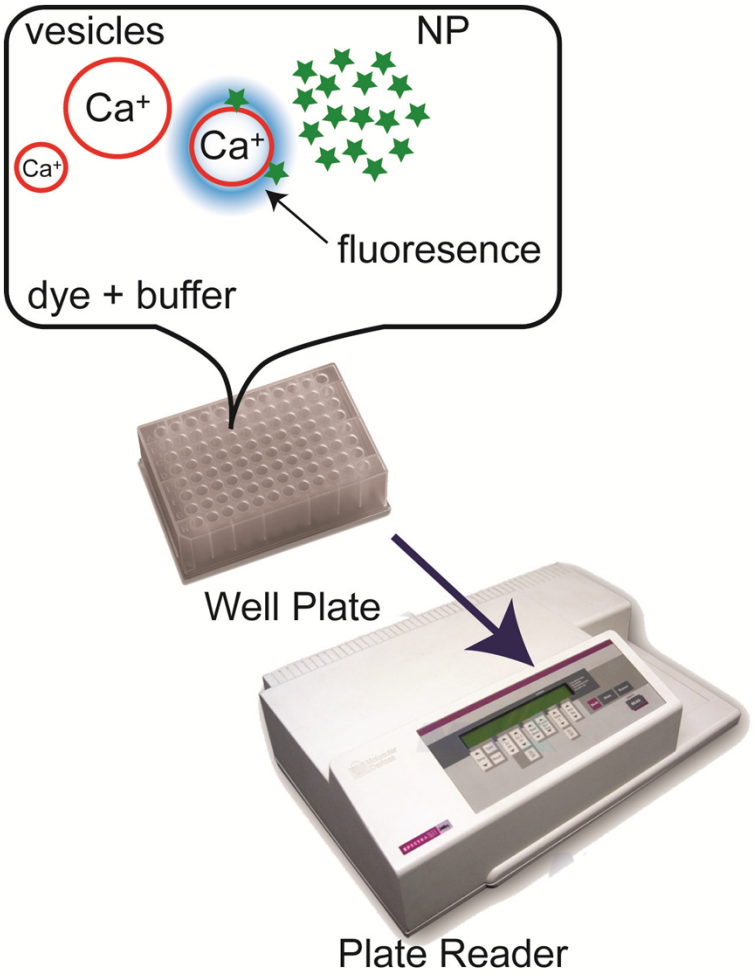
MEASURING ENM DISRUPTION OF LIPID BILAYERS USING LIPOSOMES

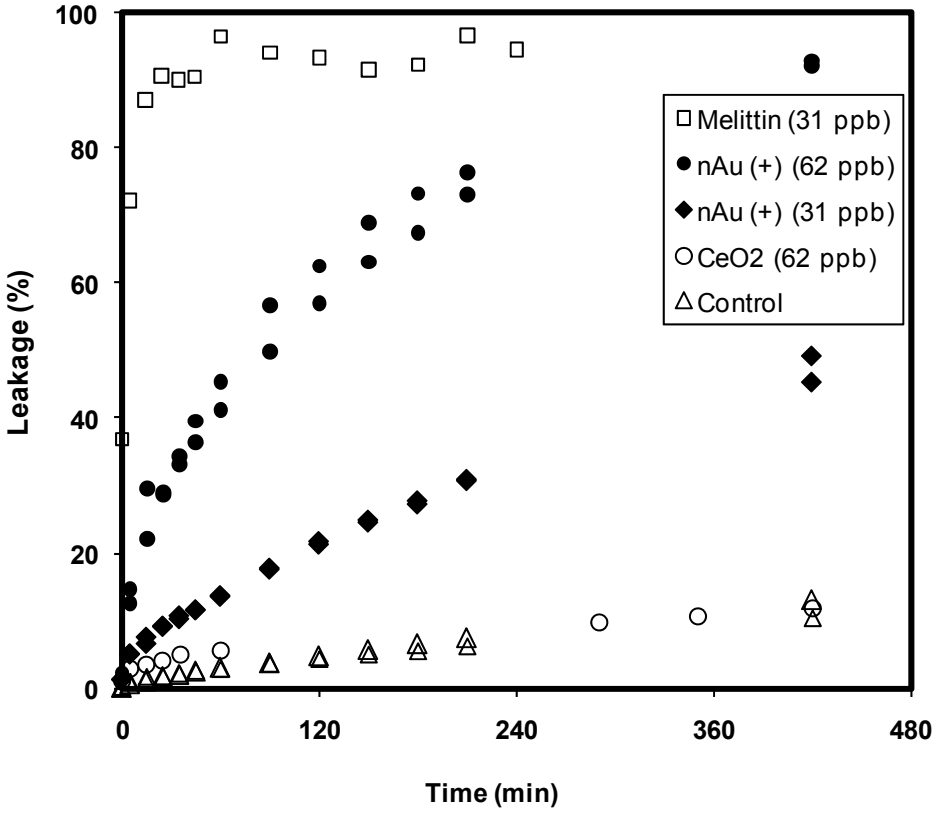


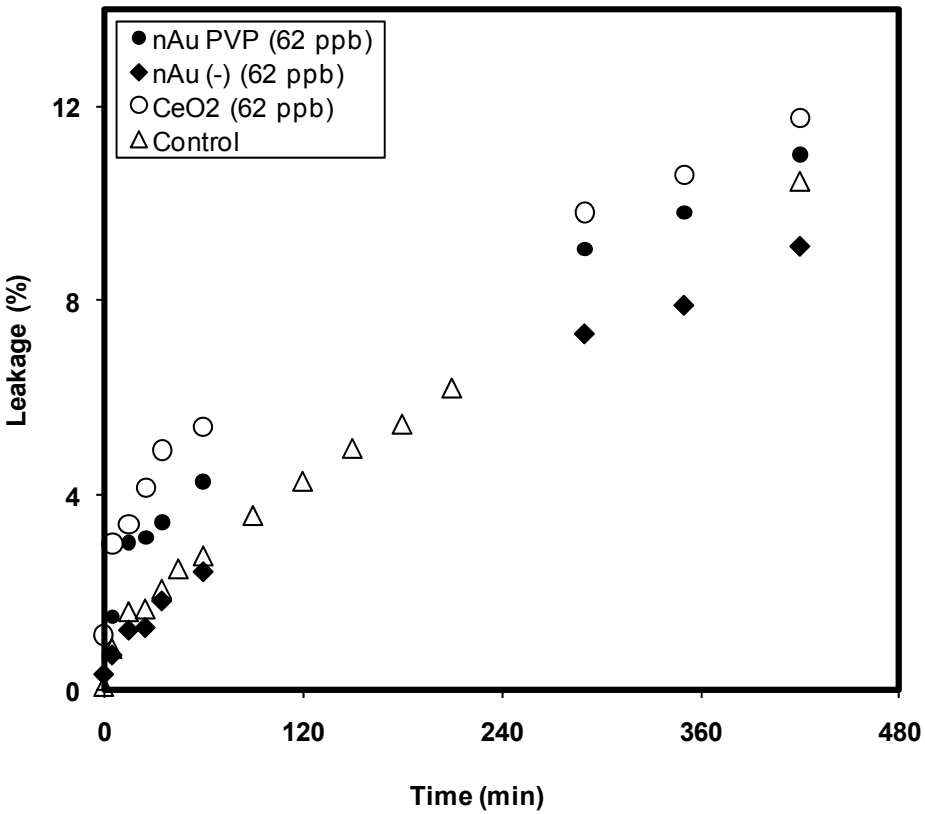
High-Throughput Liposome Leakage



- DOPC liposomes
- Carboxyfluorecein dye
- 20 mM HEPES pH 7.4
- Add NP to liposomes and stir

Leakage Kinetics





- Melittin toxin induces nearly 100% leakage in < 20 min.
- Positively charged particles induce most NP leakage
- Leakage rate increases with concentration
- Cerium induces significant leakage with no charge

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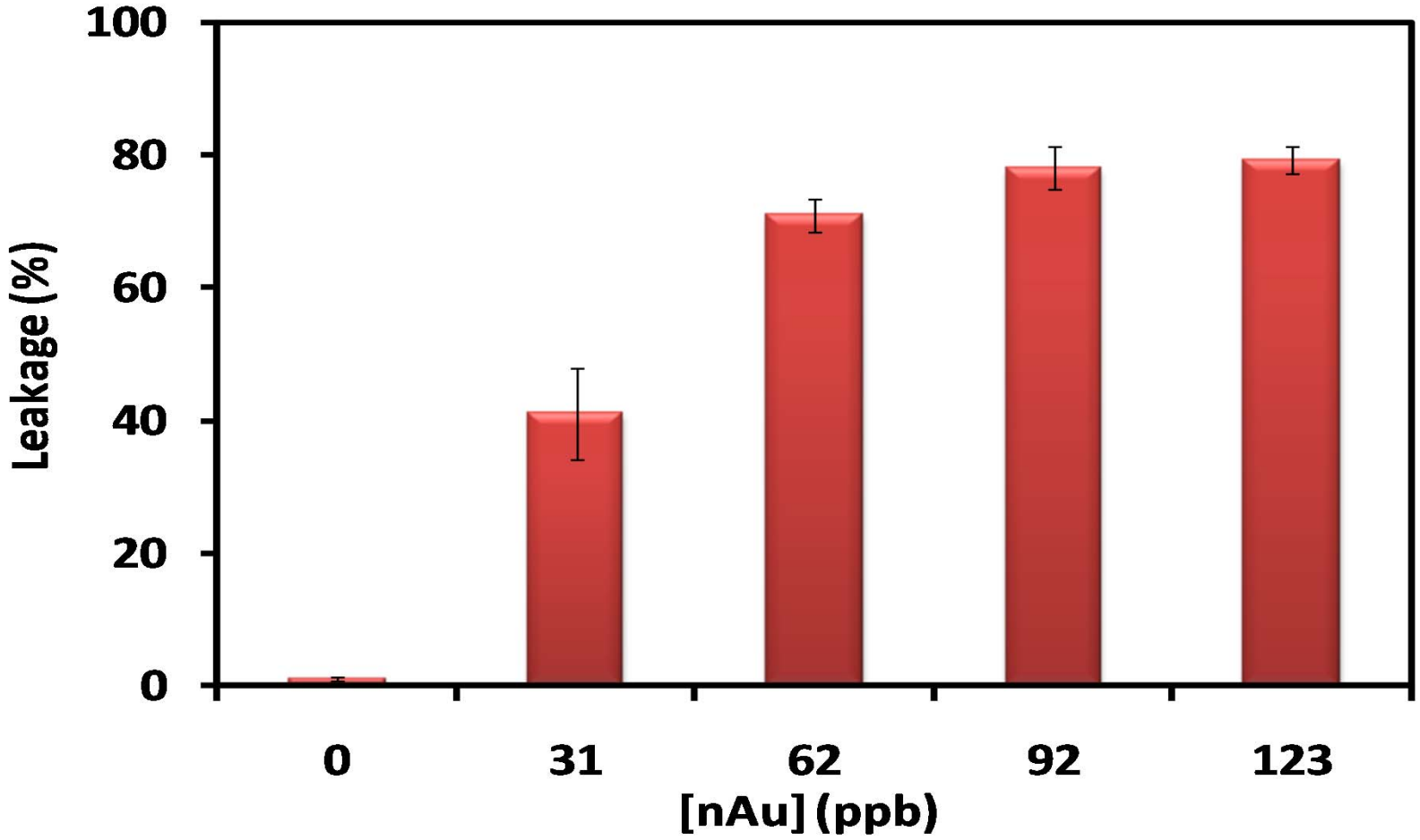
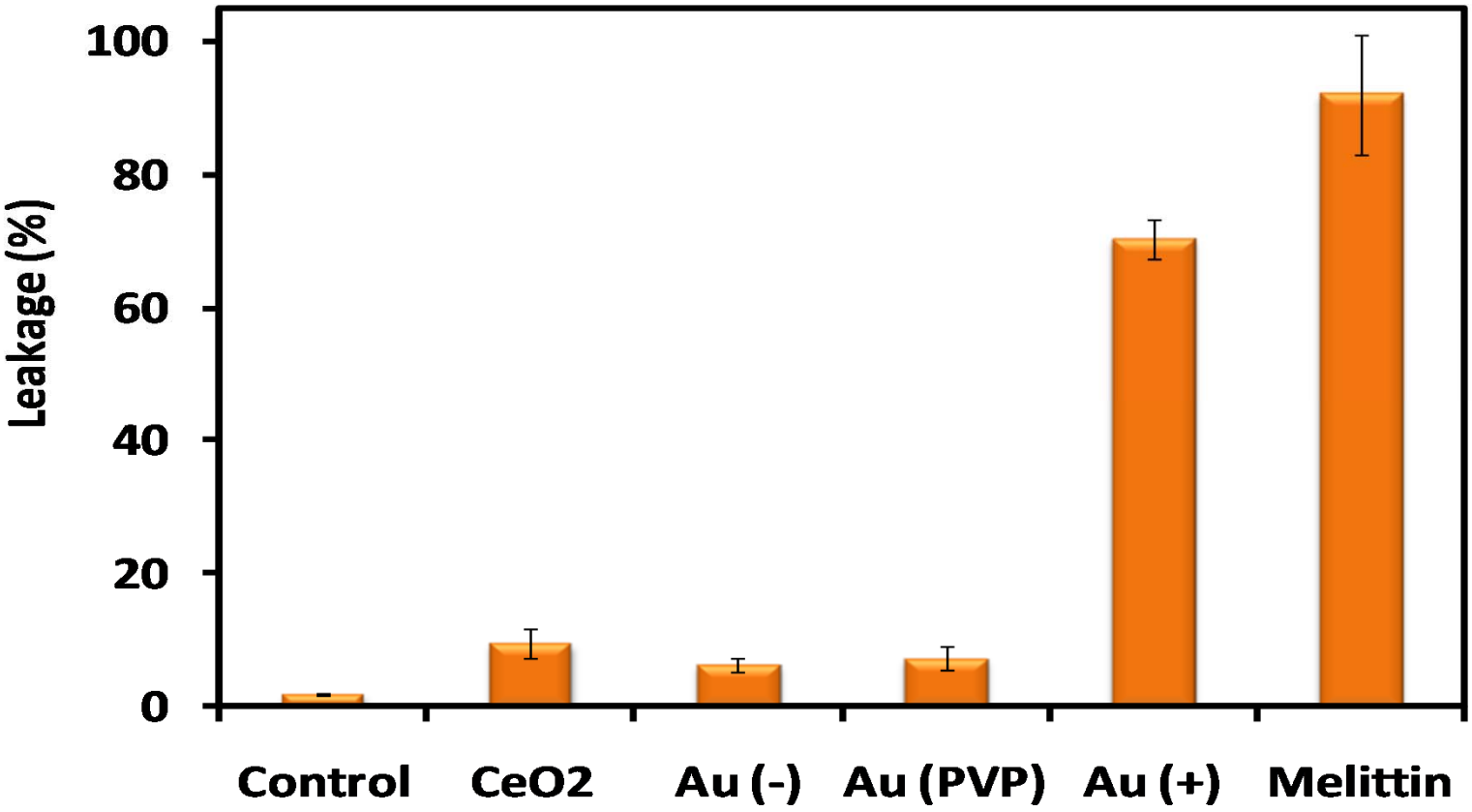
Concentration Dependence



Leakage Comparison at 31 ppb



- All particle induce leakage rough 50% of positive control melittin
- Positively charged particles induce most leakage
- Cerium induces significant leakage with no charge

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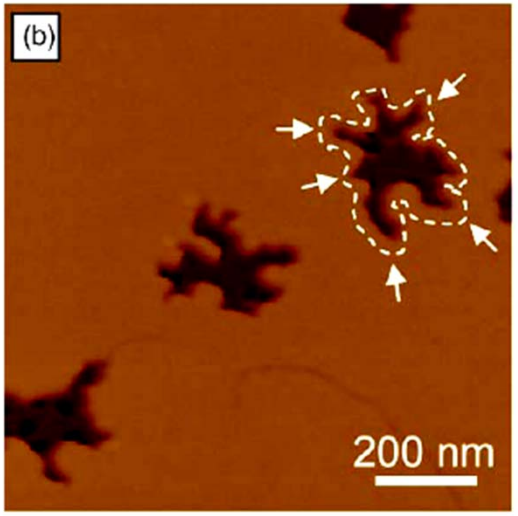
Summary

- Empirical, high-throughput global descriptors and models are needed to predict the bioaccumulation and toxicity potential of naomaterials.
- The lipid bilayer-water distribution of the selected ENMs is pseudo-equilibrium process that can be described by isotherm behaviors analogous to ionizable organic pollutants (pH dependent)
- Lipid bilayer-water distribution is promising for assessing the bioaccumulation and toxicity potentials of ENMs.
- ENM can disrupt lipid bilayers and results in leakage.
- Early reports suggest that cerium oxide does adsorb and cause significant bilayer disruption.
- Bioaccumulation (BCF) and toxicity data are needed to correlate with lipid based global descriptors to show if they can be used to predict potential hazards of NP.

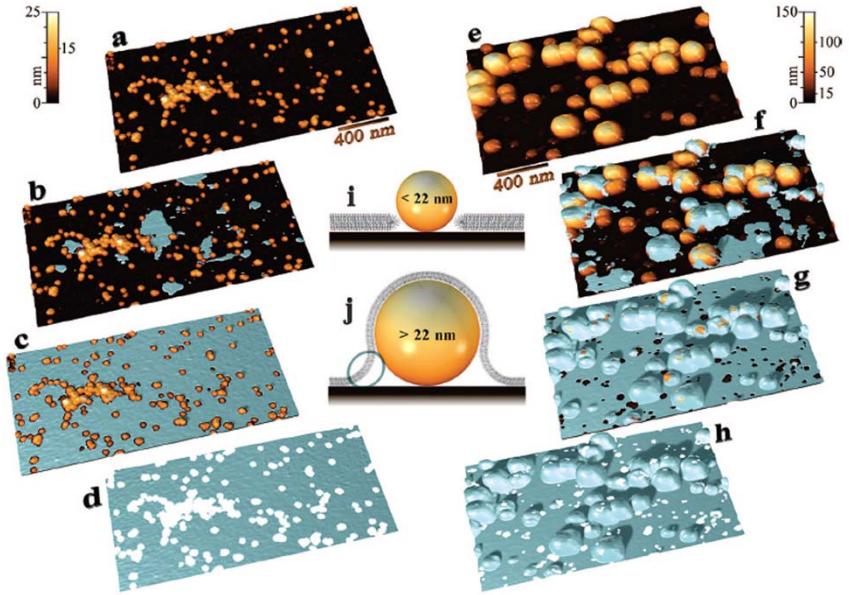
Additional Information & Backup

Existing Work on NP and Bilayers

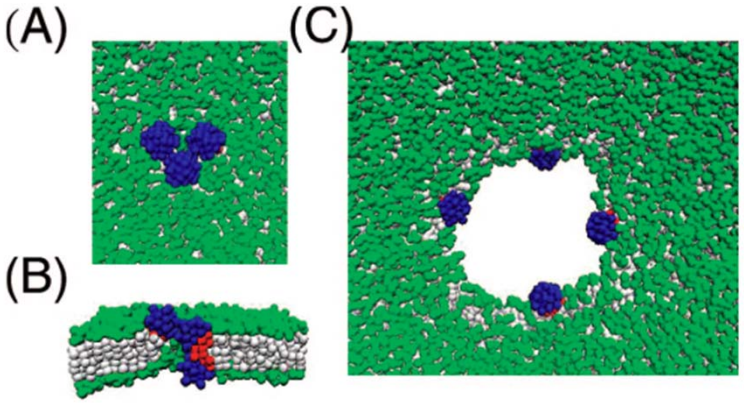
- Roiter *et al*., Nano Letters 2008
- Leroueil *et al*., Acc. Chem. Res. 2007
- Spurlin & Gewirth, Nano Letters 2007
- Alexeev *et al*., ACS Nano 2008
- Hong *et al*., J. Chem. Health & Safety 2005
- Noguchi & Takasu, Biophysical J. 2002



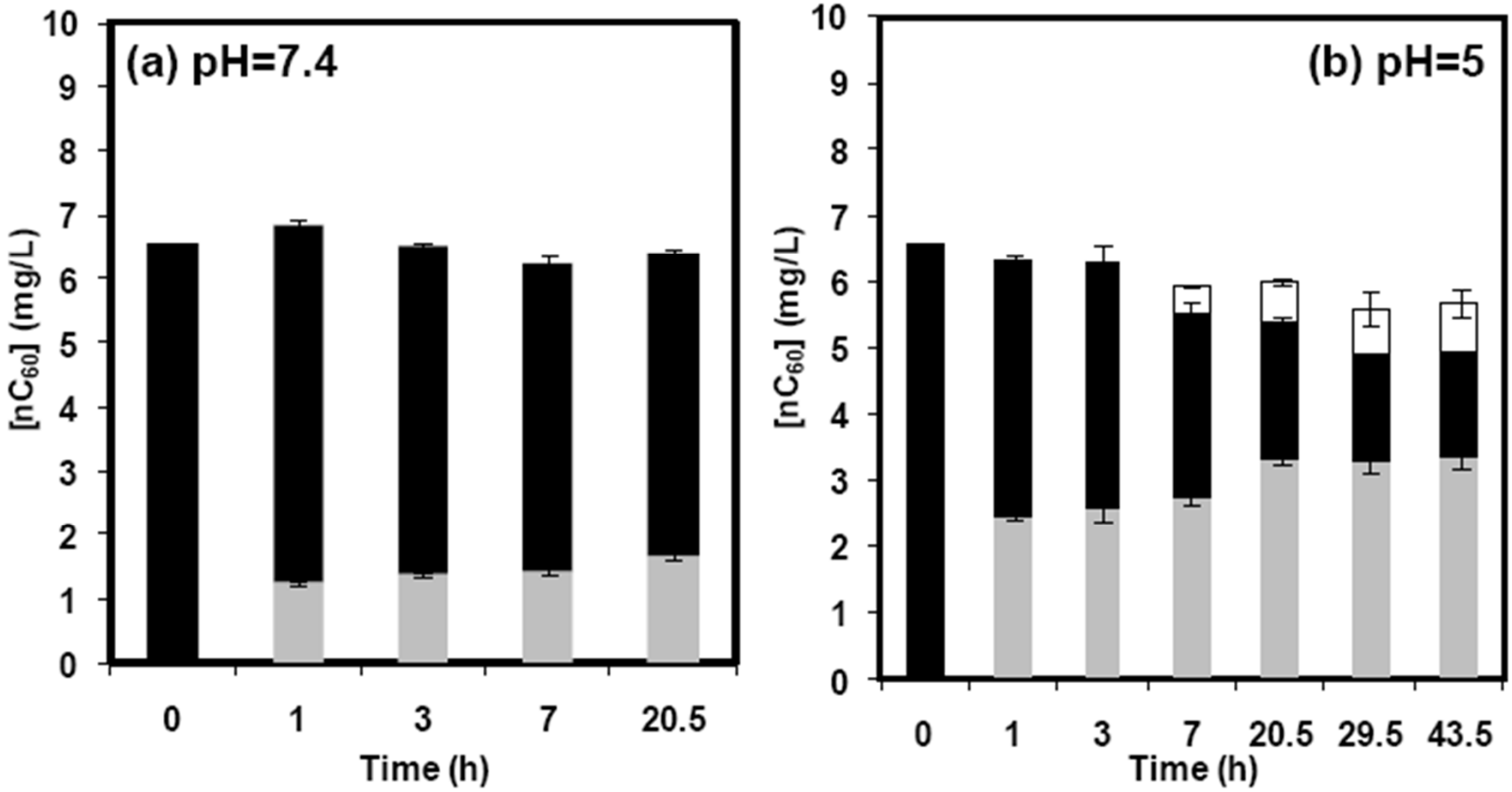
Solid Supported



Computational



nC60 Mass Balances



Black: free nC_{60} ---- Grey: nC_{60} in SSLMs ---- White: nC_{60} lost to walls

Serial ENM extraction from SSLM by original electrolyte then toluene

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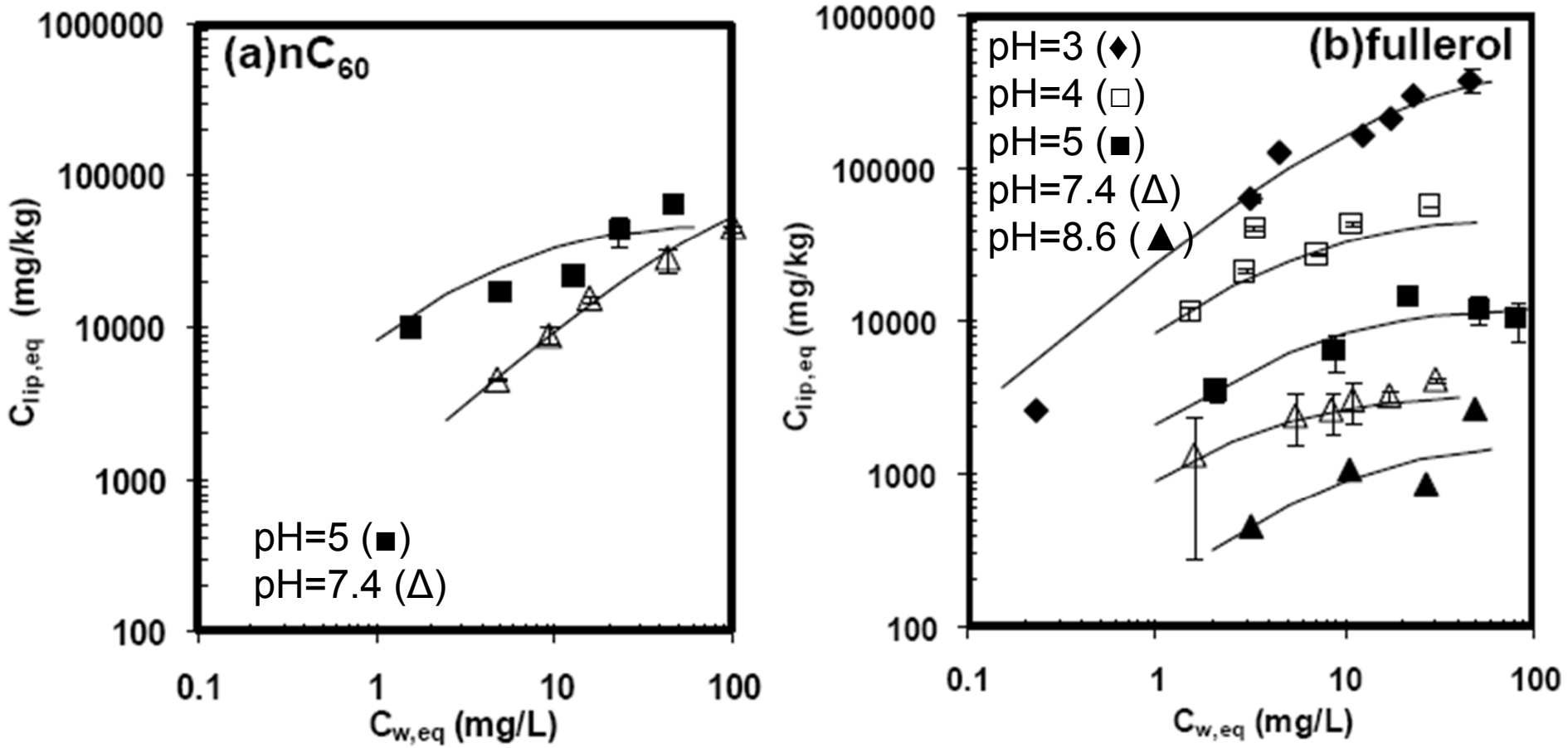
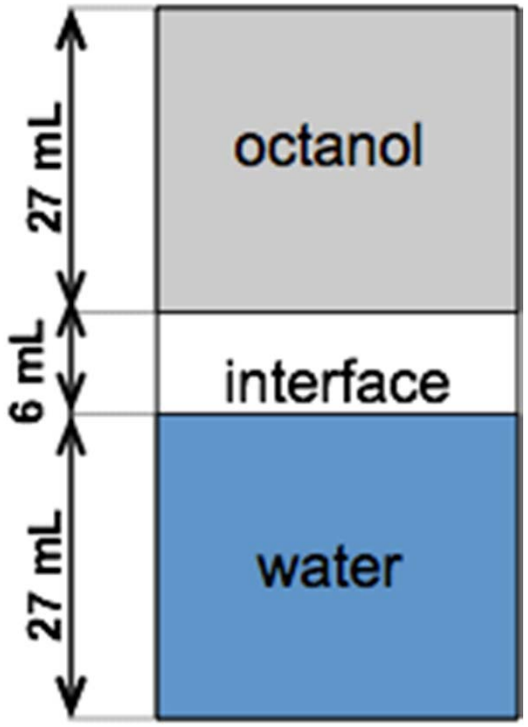
Distribution of nC60 and Fullerol in Lipid-Water

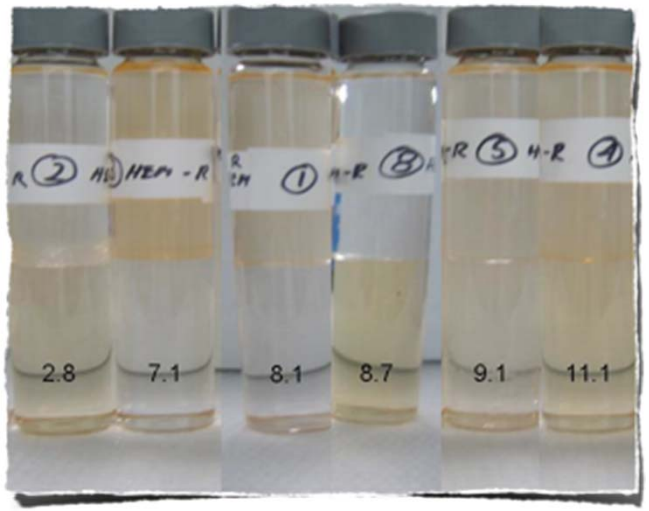


- Lines are Langmuir isotherms
- Bars indicate one standard deviation.

Octanol-Water Partitioning of ENM



Partitioning of Hematite Fe_2O_3

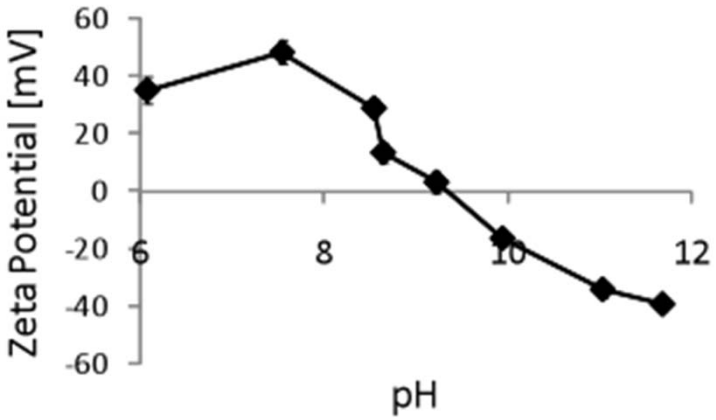


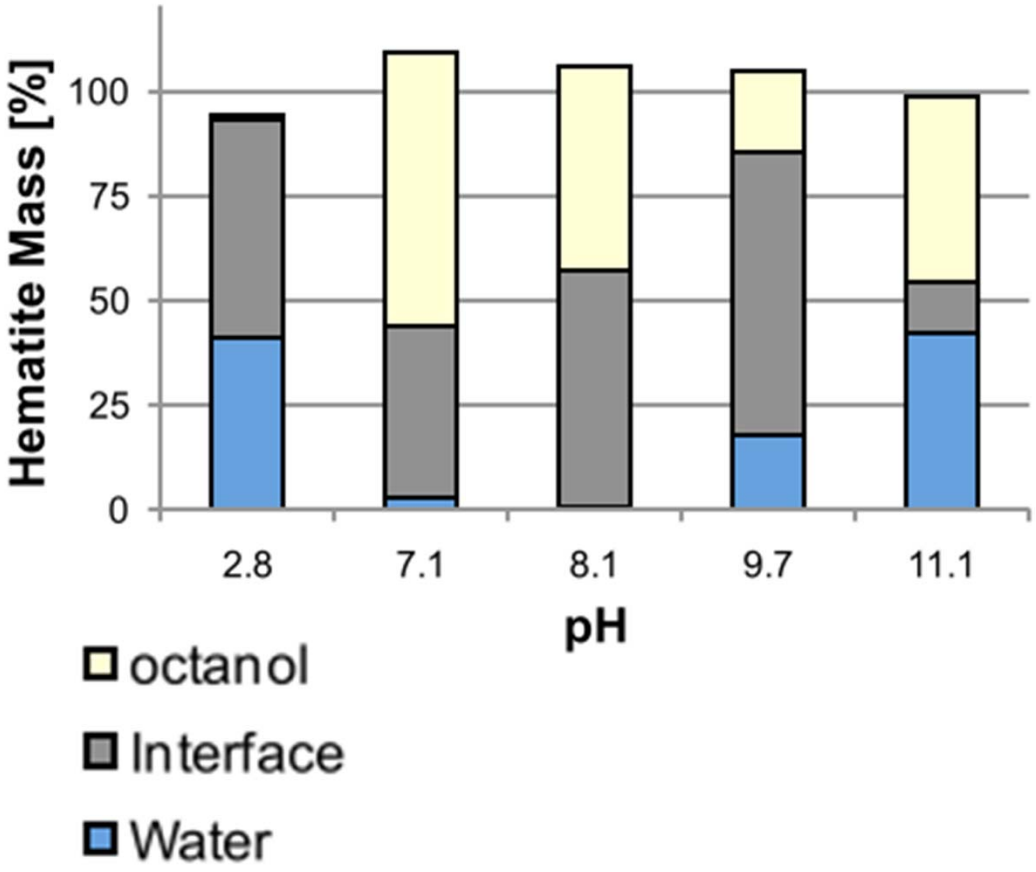
- Some particles at interface
- Minimization in Helmholtz free energy
- Not quantified or treated in classical partitioning theory

Octanol-Water Partitioning

Observe that ENM partitioning experiments result in combination of three primary, path and solution chemistry dependent scenarios:

A – in the aqueous phase
B – In the octanol
C – at interface



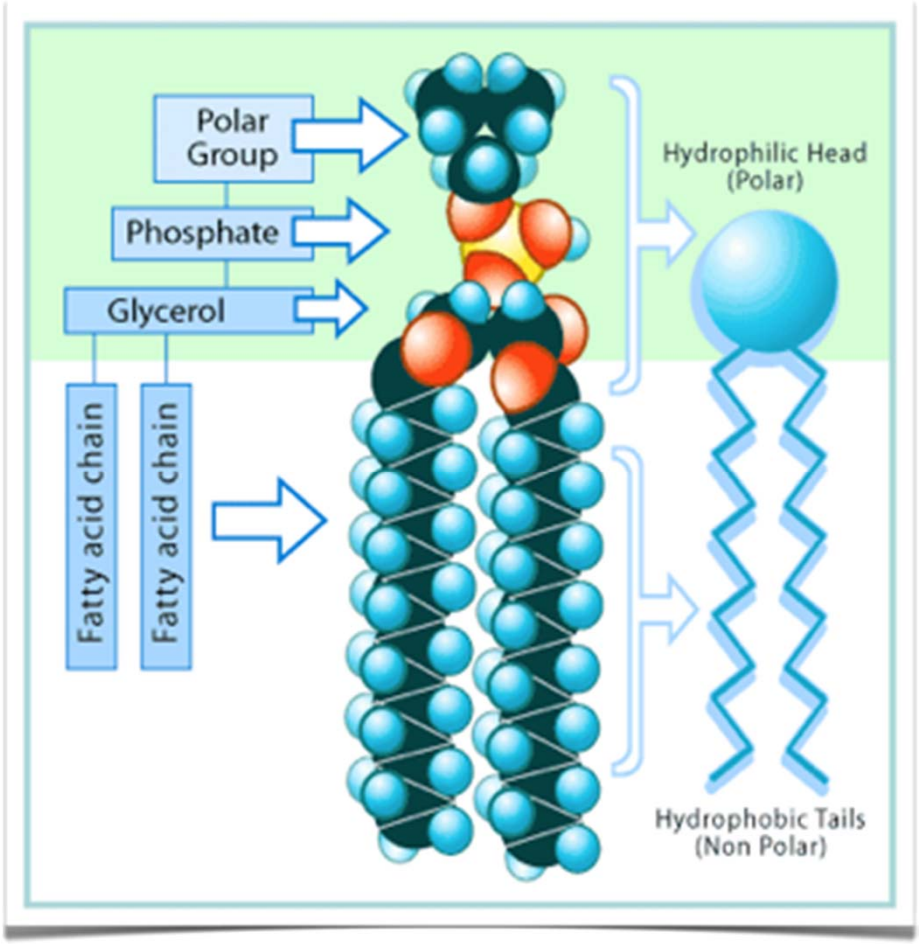


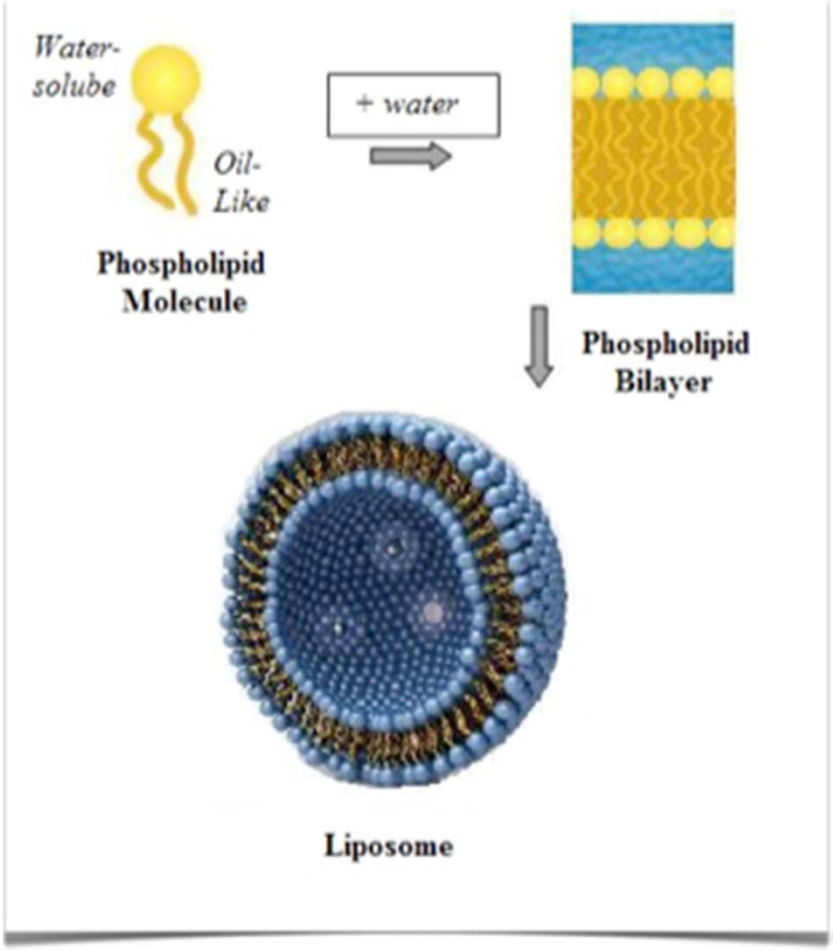
Challenges with ENM Octanol-Water Partitioning

- Importing into EPA models
- How do we treat mass at interface?
- Partitioning gives no information on state on ENM (aggregation and settled in water, dissolved, suspended, emulsion)
- Partitioning is path dependent
- Does not correlate with bioacumulation*
- Partitioning dependent on poorly defined interfacial area

*Petersen et al. Relevance of Octanol-Water Distribution Measurements to the potential Ecological Uptake of Multi-Walled Carbon Nanotubes, Environmental Toxicology and Chemsitry, 2010

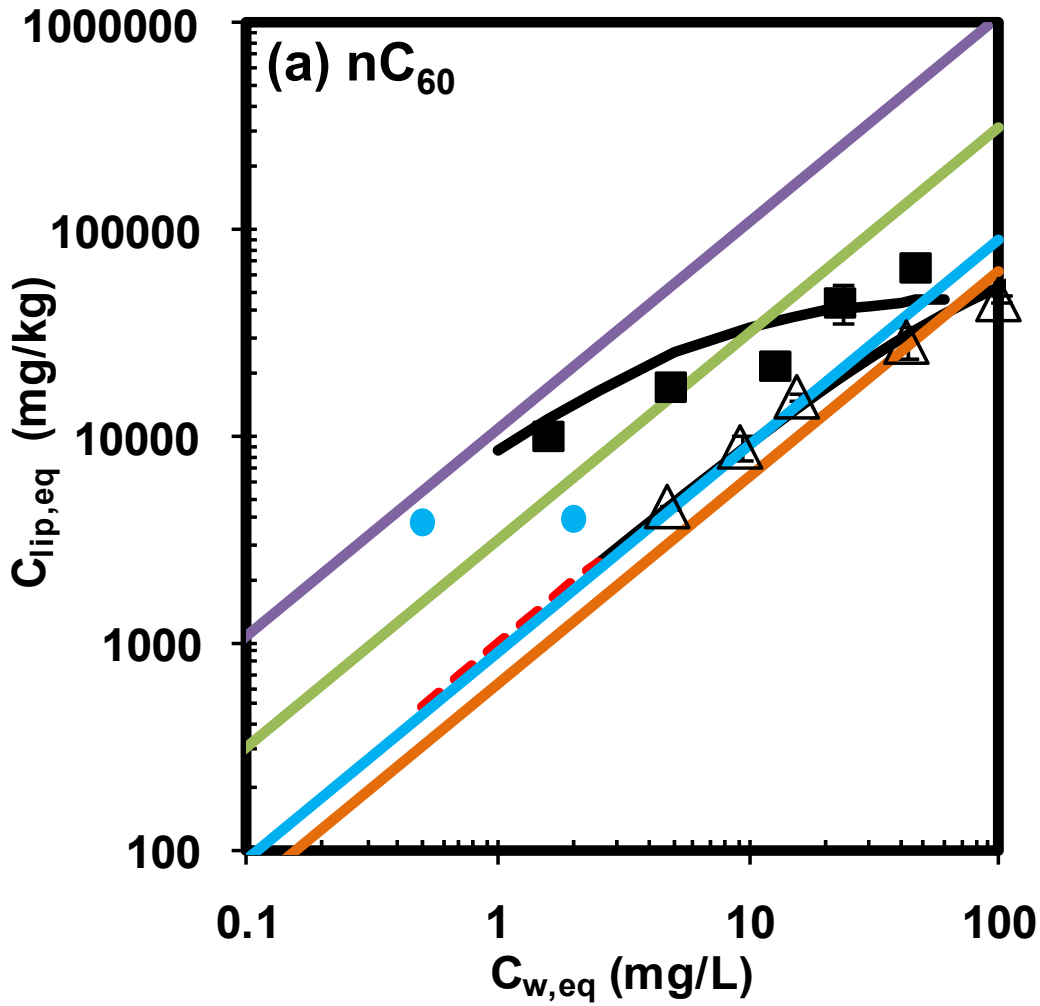
Lipid Bilayers

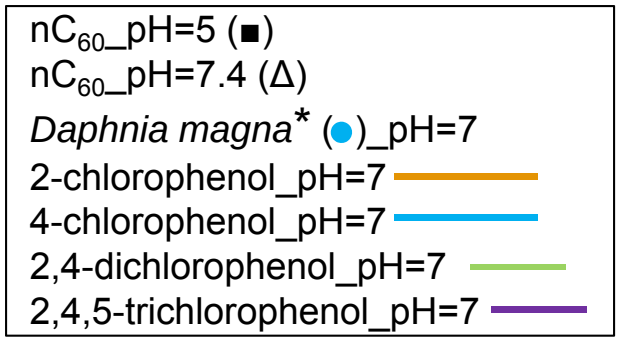




- Primary constituent of many biological cellular membranes.
- Often used to model passive transport into cells.

Comparisons with Daphnia Bioaccumulation & Ionizable Organic Compounds

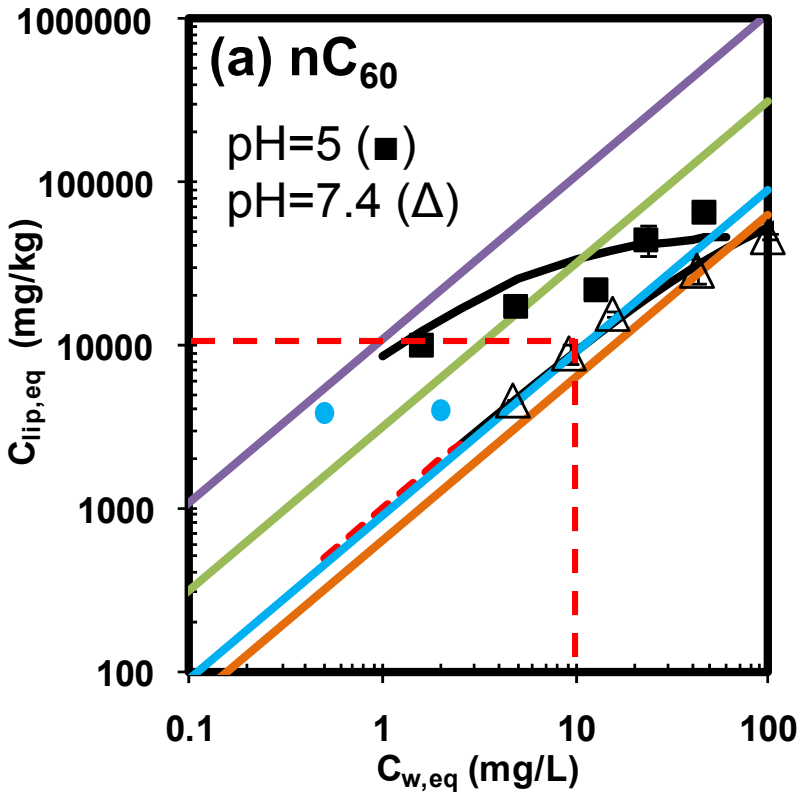


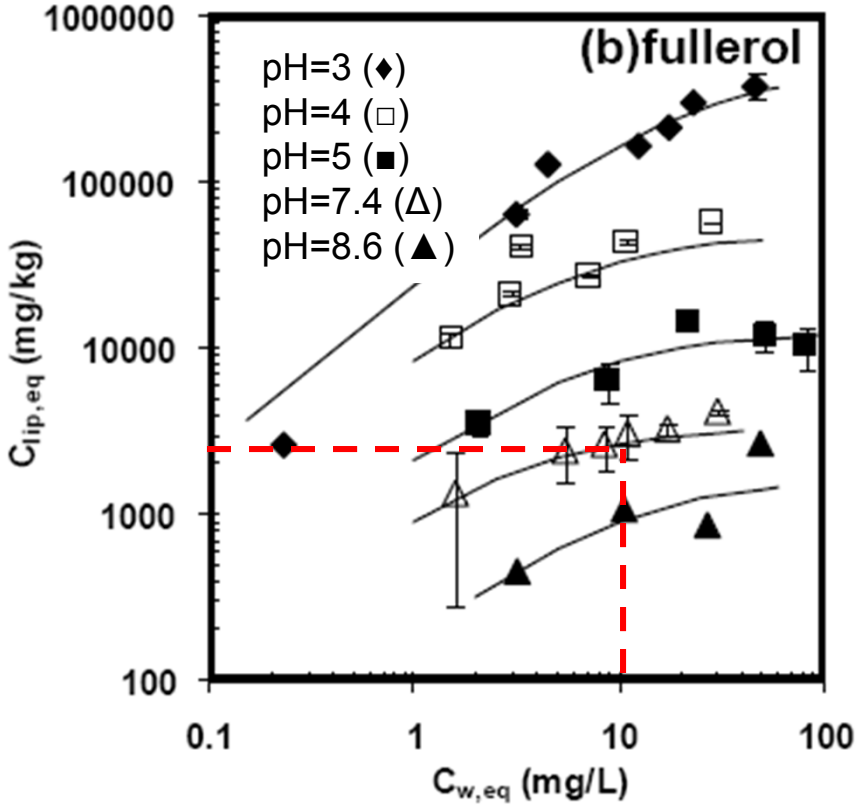


- Ionizable compound
- Show pH dependent partitioning
- Compare with chlorophenol compounds (Escher 1996)

*Bioaccumulation of nC_{60} in aquatic organisms (*Daphnia magna*) (Tervonen et al., 2010)

Comparisons with Daphnia Bioaccumulation & Ionizable Organic Compounds

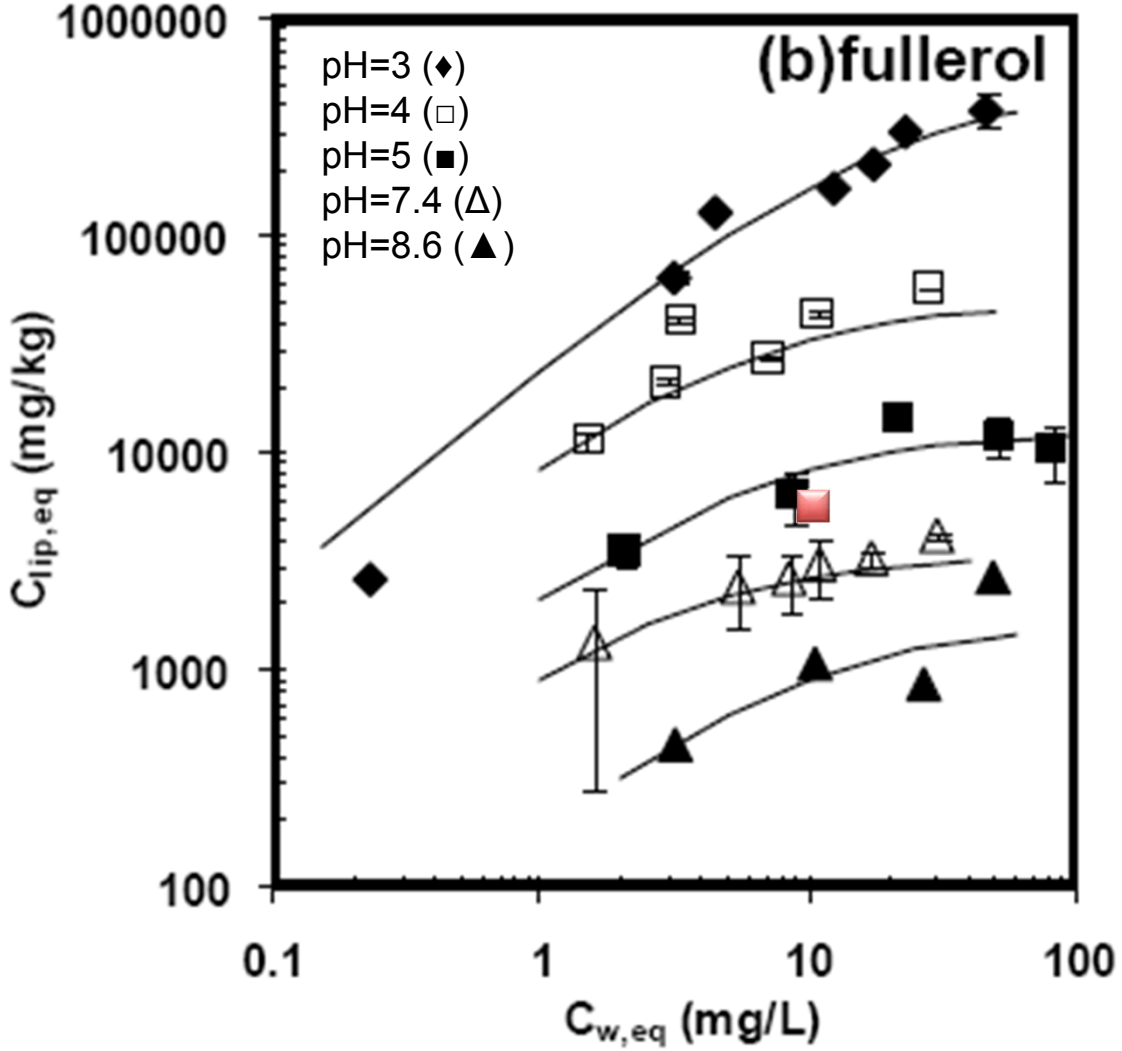




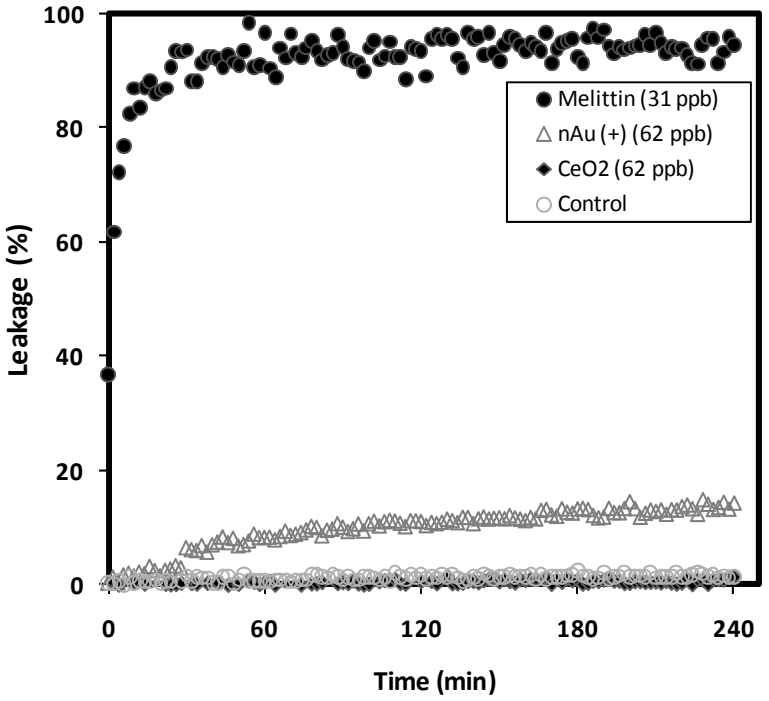
Studies suggest higher bioaccumulation and toxicity of nC_{60} than fullerol:

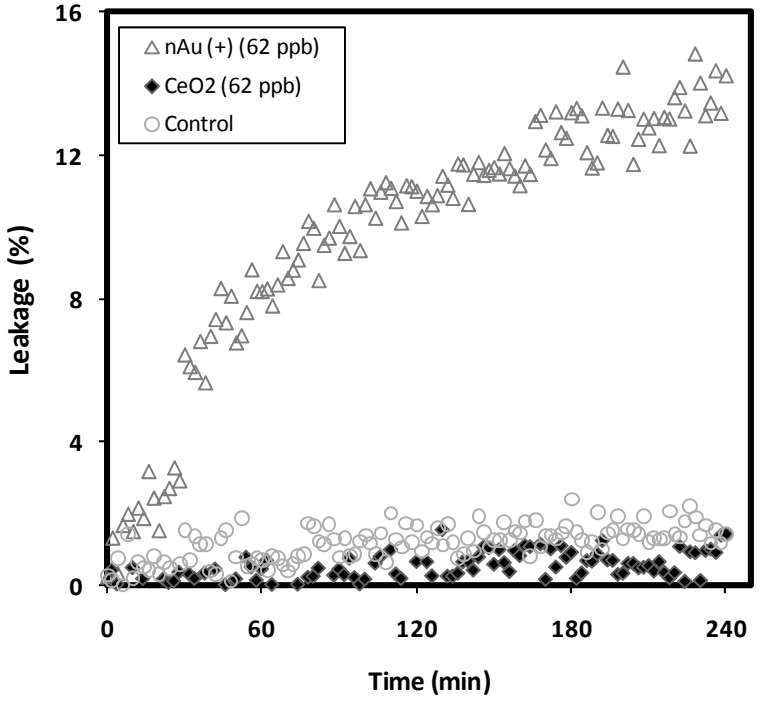
- Kiser et al., *Water Res.*, 2010. (biosorption using wastewater biomass)
- Sayes et al., *Nano Lett.*, 2004. (toxicity to human cell membranes)
- Zhu et al., *Environ. Toxicol. Chem.*, 2007. (toxicity to fish)
- Fang et al., *Environ. Sci. Technol.*, 2007. (toxicity to bacteria membranes)

Comparison between Cerium Oxide (pH=7.4) and Fullerol



No Shaking





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Cerium Zeta Potential in HEPES pH 7.4